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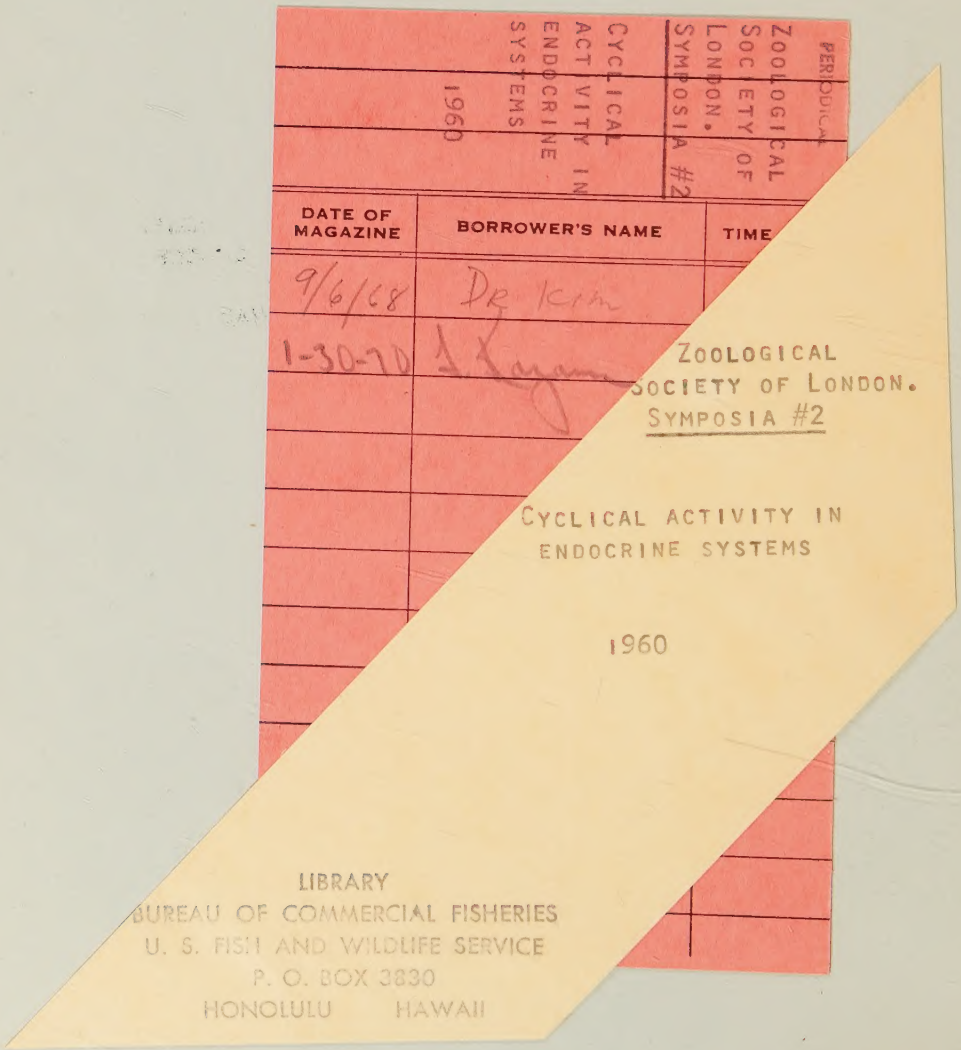
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CYCLICAL ACTIVITY IN ENDOCRINE SYSTEMS

Proceedings of a Symposium held at
The Zoological Society of London
Regent's Park, London, N.W.1
on 8th December, 1959

Edited on behalf of
The Zoological Society of London by
E. J. W. BARRINGTON

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PUBLISHED BY THE SOCIETY
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1960

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CONTRIBUTORS

Barrington, E. J. W.	Department of Zoology, Nottingham University.
Carlisle, D. B.	Marine Biological Association of the United Kingdom, Plymouth.
Marshall, A. J.	Department of Biology, Monash University, Clayton, Victoria, Australia.
Matty, A. J.	Department of Zoology, Nottingham University.*
van Oordt, P. G. W. J.	Zoological Laboratory, Department of Endocrinology, Utrecht, Netherlands.
Swift, D. R.	Freshwater Biological Association, Ambleside, Westmorland.
Wells, M. J.	Department of Zoology, Cambridge.

* Present address : The Wellcome Laboratory of Comparative Pharmacology, Gatty Marine Laboratory, St. Andrews, Fife.

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PREFACE

Few fields of biology have advanced with such success in recent years as that of Comparative Endocrinology, yet there are many aspects of it which remain much less familiar to the general zoologist than could be wished. No doubt this is to be accounted for in part by the fact that endocrinological concepts are rooted deep in specialised fields of human and mammalian physiology which are remote from the work of many students of zoology, but whatever the reason, those of us who are concerned for the further development of this subject must be very grateful indeed to the Council of the Zoological Society for devoting to it both the first and the second of its new series of whole-day Symposia.

The first one, published in June, 1960 under the editorship of Professor Chester Jones, concentrated upon a group—the fish. This one concentrates upon a problem—cyclical activity—and in so doing cuts across the limits of zoological classification to the extent of calling upon certain invertebrate groups to supplement and illuminate the results obtained from studies of the vertebrates.

Rhythms are such a profound and complex feature of living organisms that it would be wholly premature for the reader to look in this Symposium for the answers to the many questions which will be occurring to him. What he will, however, find are accounts of work in progress, and I am confident that he will be fascinated by the intricate nature of the problems with which the speakers were concerned.

Although I imagine that A. J. Marshall will not wish us to take him too seriously when he says that the study of avian breeding seasons will soon cease to be an endocrinological problem, he is profoundly right in insisting that they are the business also of the ecologist, ethologist and neurophysiologist. It is, indeed, peculiarly the value of Comparative Endocrinology, both as a training ground for zoologists and as a source of material for their future investigations, that it ramifies into so many branches of the biological sciences and, in so doing, forces us to try to develop an integrated picture of the animal in relation to its environment at all stages of its life-history.

I hope that something of the exciting character of the subject, and of the breadth of its perspectives, will become apparent to those readers of these pages to whom the problems are unfamiliar, and I hope that it may induce some of them to commit themselves to it as a field of research. Certainly those of us who contributed to the Symposium would regard such a result as our best possible reward, but at the same time we shall also hope that other endocrinologists will find it helpful to have these reviews of our work set out in the convenient format which has been made possible by this new development in the activities of the Zoological Society.

E. J. W. Barrington

July, 1960

THYROID CYCLES IN FISH

BY

A. J. MATTY

*Department of Zoology, Nottingham University**

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INTRODUCTION

Cycles of endocrine activity are common phenomena and the thyroid gland of fish is no exception, but whereas in some glands, for example the gonads, both causes and effects of these periods of recurring activity are well established, the reasons for changes in fish thyroid activity are not so well known. This is not so surprising for in spite of many investigations there is little evidence for the exact functional role of the fish thyroid and at another recent symposium it was said of this gland that " the peripheral target of the hormone is unknown and so is its physiological effect " (Berg, Gorbman & Kobayashi, 1959). This, indeed, is an unsatisfactory state of affairs for the comparative endocrinologist. However, one way of furthering our knowledge of the gland might be to review and analyse its cyclical activities along with some experimental observations. This I propose to attempt. As the subsequent paper in this symposium will be concerned with the fish thyroid and the environment, I shall not attempt to review this, but to concern myself mainly with the probable relationship of the thyroid in fish with reproduction and some probable endogenous factors influencing the gland.

EVIDENCE FOR CYCLICAL ACTIVITY IN THE THYROID OF FISH

Most of the evidence for cyclical activity in the fish thyroid comes from histological and cytological investigations made seasonally or during the period of metamorphosis. Those fish which undergo a metamorphosis and during which a change in thyroid activity is observed might here be thought of as exhibiting a cycle which only occurs once in the life of the animal.

Qualitative studies of the histological seasonal changes exhibited in the teleost thyroid were begun a number of years ago when activity was inferred from the size and number of cells and amount of colloid in the follicles. Lieber in 1936 examined *Misgurnus fossilis* and found that from the beginning of October to the end of March the thyroid was in a condition of greatly reduced

*Present address : The Wellcome Laboratory of Comparative Pharmacology, Gatty Marine Laboratory, St. Andrews, Fife.

activity ; in April there was an increase in activity which continued until June when a decreased activity became apparent. Finally in August a second upsurge of activity appeared. These changes were thought to be largely due to seasonal changes in feeding. Hagen (1936) described a similar series of changes in the eel. Hoar (1939) made a detailed examination of the thyroid of the Atlantic salmon, and in the course of this also described seasonal variations. There were most signs of activity in early spring, a period of mild activity from June to November and a period of quiescence in the winter. There appeared to be no correlation of these changes with sexual maturation. Buchman (1940) noted during the pre-spawning period of *Clupea harengus* colloid storage in the thyroid whereas after spawning there occurred a period of increased cell height and colloid discharge. Qualitative estimates such as these are difficult to interpret as all observers when examining the teleost thyroid have found wide variation in the form of the follicles and the follicle epithelium. Barrington & Matty (1954) made a quantitative estimation of the thyroid gland of the freshwater minnow *Phoxinus phoxinus*, by measuring the follicle epithelium height at monthly intervals during the year, it being thought at the time that cell height, as in mammals, would provide a useful criterion of activity. These results expressed as micrometer eyepiece units (0.01 mm. = 121 units) are shown in Fig. 1. Since this time there have appeared several more quantitative estimates of changes in the thyroid epithelial height of fish. Smith (1956) in the brown trout *Salmo trutta*, found similar changes to that described by Hoar (1939) for the Atlantic salmon ; that is there was a continual increase in epithelium height from January until April and then a gradual fall until September when it remained fairly constant until January. Smith found no

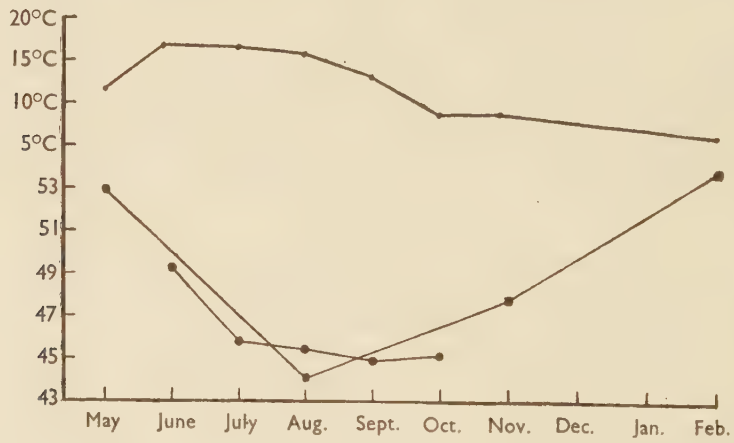


Fig. 1.—Seasonal variation in the height of the follicular epithelium of the thyroid gland of the minnow.

Upper curve : Temperature of surface water (—●—)

Lower curves : Mean cell-heights of monthly samples in 1950 (—■—) and in 1952 (—●—).

Abscissae : Months (May to February).

Ordinates : Temperature in dregrees Centigrade (above) and cell-heights (below).

(After Barrington & Matty, 1954).

significant difference between the thyroid cell heights of mature and immature males nor between male and female immature fish. He did not apparently make any comparison between mature male and female fish. Swift (1959) also described a similar cycle for yearling brown trout (see subsequent paper of this symposium), while a Japanese salmonid, the ayu *Plecoglossus altivelis*, has also been a subject of a quantitative study by Honma (1959). This latter author again found that the peak epithelium height occurred during April and May, and fell abruptly to a minimum height at the end of June. The only quantitative estimation of follicle epithelial height in a totally marine fish has been made recently by Woodhead (1959a, b). The fish investigated was the cod *Gadus callarias*, and both the mature and immature fish go through seasonal cycles, but unlike the brown trout it is the mature form which shows the greatest change in follicular cell height.

The investigations have shown that the seasonal difference in epithelium height is much greater in the salmonids, being of the order of several hundred per cent, than in the minnow or the cod where the change is much less than a hundred per cent. Also the most rapid change occurs in the ayu as it ascends the river from the sea.

These histological demonstrations of seasonal changes in the teleost thyroid have recently been supplemented by the use of radio-active iodine. Radio-iodine may be used to determine quantitatively the rate of iodine uptake or discharge from the thyroid, hormone production by the gland or the rate of hormonal release into the blood. The work of Swift (1959) using I^{131} is described

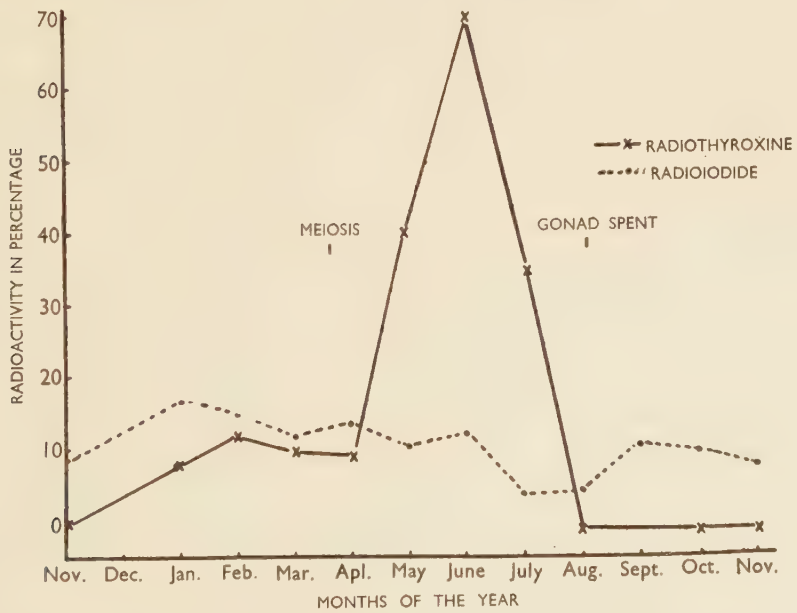


Fig. 2.—Maximal radioiodine uptake and maximal radiothyroxine production in *Fundulus heteroclitus* during eleven months of the year. (Reprinted with permission from Gorbman, *Comparative endocrinology*, 1959, John Wiley & Sons Inc.).

by himself in the following paper in this symposium, but the result of Berg, Gorbman & Kobayashi (1959) needs to be reviewed. These workers treated *Fundulus heteroclitus* with I^{131} and after a period of time measured the uptake of total iodine and of radiothyroxine. It was found that thyroidal I^{131} uptake varied with the season, being four times as great in January as in July. Also, as is seen in Fig. 2, they found that there was a seasonal cycle in the production of I^{131} labelled thyroxine, a marked peak being reached in June, falling to zero in August. The cycle of thyroidal I^{131} uptake was not well correlated with seasonal variation in thyroxine production, thyroid epithelial cell height or with photoperiod. Thyroxine production, however, was well associated with the time of reproduction and furthermore it was found that individual *Fundulus* that had the highest thyroxine production were actively breeding while those with quiescent thyroids had spent gonads.

No seasonal quantitative studies of the type described above for teleosts have been carried out on cyclostomes, largely, no doubt, because of the difficulty of obtaining natural populations of these animals throughout the year. Lanzing (1959), however, has examined the thyroids of migrating *Lampetra fluviatilis* throughout the whole migratory period. He found no distinct histological differences between the glands of the lampreys caught early in the migratory period and those of the fully mature animals, neither did he note any differences between thyroids of male and female. Apparently the qualitative picture during migration was one of a mildly active gland, with the follicle epithelium containing droplets and globules and the lumen very small amounts of colloid. An active thyroid judged on these criteria has also been observed for the permanently marine cyclostome *Myxine glutinosa* (Matty, unpublished). Again no seasonal quantitative study of the elasmobranch thyroid has been made and the bulk of data on this gland has been of an observational nature, relating thyroid function to reproduction. This will be discussed later.

Few observations have been made to indicate naturally occurring changes in the fish thyroid of a non-seasonal nature. Stolk (1951), using the histological method of Lever (1948), demonstrated that in the viviparous cyprinodont *Lebistes reticulatus*, there existed a distinct relationship between the thyroid gland and the gestation period. The gland reached its maximum activity during the second and third week of the gestation period coinciding with the phase of rapid embryonic growth and differentiation. The same author (Stolk, 1956) has also reported a similar series of changes occurring in the halfbeak *Dermogenys pusillus*. Here, then, is an indication of thyroid activity which is clearly independent of the time of year at which it occurs. Day to day variation in thyroid activity appears to take place in the eel *Anguilla anguilla*. Eels have been injected intraperitoneally with carrier-free I^{131} and kept in aquaria for fourteen days. The radio-activity of the thyroid region has been measured in a manner similar to that described by Swift (1955) at the same time each day for six days. Fig. 3 shows the variation in count rate that occurred over this period. The temperature remained constant during the period of these experiments.

I wish now to comment upon the methods of approach that have been used to record thyroid activity in fish and to mention some of their shortcomings.

I have already referred to the use of I^{131} for thyroid destruction, and it is here obvious that the generalized injurious nature of the radiation must be considered carefully, particularly when the state of the gonads is being investigated. Similarly the use of goitrogens is probably not a wise procedure when endeavouring to establish a relationship with gonad activity, for a number of such antithyroid drugs have been shown to have a general depressant effect on metabolism. Thiourea has been shown to inhibit oxygen utilization in all organs except the heart and to act as a general cell antioxidant (Reid, 1953). It seems reasonable to suppose that actively dividing cells such as those of the gonads might be the first to be effected by a metabolic inhibitor (see also below).

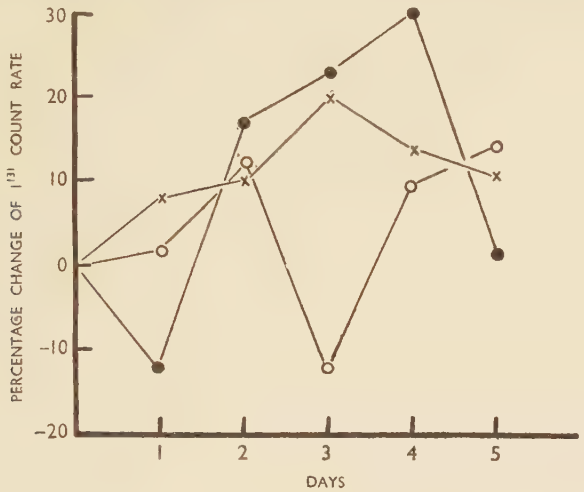


Fig. 3.—Daily variation of concentration of I^{131} in the thyroid of *Anguilla anguilla*.

One must also suspect inferences drawn from the measurement of thyroid epithelium height alone, for, as Swift (1959) has shown, epithelium height and radio-active iodine output by the thyroid are not necessarily correlated. Also Fontaine, Leloup & Olivereau (1952) have correlated the histology of the salmon parr, parr-smolt and smolt with the I^{131} uptake in these forms and found that iodine turnover was less in the smolt than in the parr-smolt whilst the former was histologically the more active gland. Can we, then, be sure that a gland with a high epithelium is producing and releasing as much hormone into the blood as a gland with a not so high epithelium? It may seem unnecessary to point this out, but it is a fact that many investigations of the fish thyroid have made use of epithelium height as the sole criterion when relating the gland to a probable function. In the past this was almost the only method of assessing thyroid condition in fish, but it has added very little to our knowledge of the physiology of the gland. Hoar (1959) has recently stated that much of the work which has endeavoured to correlate the thyroid with an osmoregulatory and a migratory function using histological hyperplasia and hypertrophy of the thyroid alone as a measure of activity may in fact only have indicated the response of the thyroid to an iodine-deficient medium. The great change in epithelial height described for the ayu by Honma (1959), which

occurs in May as the animal migrates into the rivers low in iodine content, could be explained on this basis. Hoar has pointed out that the seasonal thyroidal hypertrophy and hyperplasia which have been described for other anadromous fish in fresh water may perhaps now be regarded as being goitrogenic rather than physiological. Evidence for this viewpoint comes largely from the work of Hickman (1958) on the starry flounder *Platichthys stellatus*. In this fish it was shown that the thyroid radio-active iodine uptake was greater in fresh than sea water, but when fish were placed in fresh water containing the same amount of iodine as sea water, then the uptake was in fact greater in the marine fish. But even this excellent work of Hickman when it is used to try and understand changes in thyroid hormone activity must be regarded with care for one has to remember the results of Berg, Gorbman & Kobayashi which show that iodine uptake and thyroxine production do not necessarily show any relationship in fish. Thyroids of *Oncorhynchus garbuscha* and *Oncorhynchus keta* fry which are retained in fresh water beyond the time of migration and fed on beef liver are hyperplastic, but if such fry are fed on a diet of marine fish, then hyperplasia fails to appear, again indicating that this hyperplasia is goitrogenic in origin (Hoar, 1959). In the light of these investigations it would probably be worth while if future studies of thyroid activity used measurements of the hormone content of the gland, hormone or iodine output by the gland, and hormone content of the blood, rather than relying on histological data alone.

Although low iodine content of fresh water may account for apparent changes of thyroid activity in anadromous migrants, it certainly cannot for katadromous migrants. Nor, except perhaps in the case of the eel *Anguilla*, does a change in iodine content influence the changes seen in the thyroid of larval teleosts which undergo metamorphosis, for the process of transformation appears to occur in a constant environment. Pickford & Atz (1957) have reviewed the literature which indicates thyroid changes at metamorphosis, and also the probable pituitary control of these changes. The picture is far from clear, for thyroid activity on occasions appears to precede pituitary cellular differentiation, both in teleost and acipenserine fishes. The postulation of an inherent, or non-pituitary mediated, thyroid variation is easy to make but difficult to substantiate.

THE THYROID AND REPRODUCTION

From the investigations that have been described it appears that many, but not all, of the changes that appear in the teleost thyroid are related to reproduction, for example spawning occurs just after the period of greatest histological activity both in *Phoxinus* and *Gadus*. The reproductive cycle sets much of the physiological background of fish and the thyroid may well become involved. Pickford & Atz (1957) have reviewed the literature which has demonstrated in a qualitative manner changes occurring in the thyroid during the period of reproduction, particularly that of the Russian workers (S. A. Ivanova, 1953 ; A. D. Ivanova, 1954 ; Zaitzev, 1955 ; N. L. Gerbil'skii, various publications). These authors working with various species of *Acipenser* and with *Esox lucius* have observed marked changes occurring in the thyroids during

spawning, not only in epithelium height but in colloid content. Laboratory experiments have seemed to confirm this relationship between thyroid and reproduction ; for example, the maintenance of fish in anti-thyroid drugs has inhibited the maturation of the gonads. Chambers (1953) found that the testes of *Fundulus heteroclitus* kept in a solution of thiourea regressed and similar results were achieved by Barrington & Matty (1952) on the minnow *Phoxinus phoxinus*, and by Smith, Sladek & Kellner (1953) on *Lebistes reticulatus*. Furthermore, another form of thyroid inhibition has been performed by Baker, Berg, Gorbman & Gordon (quoted in Pickford, 1957) in which the thyroids of *Xiphophorus* were destroyed by I^{131}. Here again it was found that the maturation of the gonads was retarded. Such results must, however, be interpreted with the greatest of caution for subsequent experiments on the minnow by Professor Barrington and myself using a different anti-thyroid drug, thiouracil, did not inhibit spermatogenesis. The conditions of the experiments were not identical, for whereas the minnows treated by thiourea were immersed in the solution from January to March, the minnows immersed in thiouracil were treated for two months from October to December under conditions of artificial light. Nevertheless, although the thyroids were completely hypertrophied and the colloid discharged, complete spermatogenesis took place ; in fact, when males of approximately the same size were compared it was found that eight out of fourteen of the testes of the thiouracil fish contained spermatids whereas only one out of twelve of the control fish testes contained spermatids. Although surgical thyroidectomy has been performed on both elasmobranch and teleost fish (Matty, 1954, 1957) no obvious effects on the gonads have been noted, but this may be due to the fact that the periods of thyroidectomy were too short, or that the experiments were conducted at a period of the year when the thyroid was not influencing the gonads. Again, it must be remembered that in *Clupea harengus* Buchmann (1940) found that the increase in follicle epithelium height and the discharge of colloid took place *after* spawning, and that the period of spawning of the salmonid fish that have been examined occurs many months away from the months of highest epithelium height. Certainly the correlation of thyroid activity with reproduction is not a simple task.

The cyclostomes and elsamobranchs have not been so widely investigated as the teleosts and in fact there is no indication at all that there exists any relationship between thyroid and reproduction in the former, except for the solitary observation of Leloup (1952) who found a higher uptake of radio-iodine in the male *Petromyzon marinus* than in the female. In the elasmobranchs, however, there is more evidence, and this has been reviewed by Olivereau (1954). The thyroid is hypertrophied during the period of growth of the gonads and in the females of certain oviparous species there is also activity during the period in which the eggs are in the shell gland ; furthermore, the thyroid is hyperactive in live-bearing species during gestation. Leloup (1949a b, 1951) has measured the blood iodine, blood copper and thyroidal fixation of I^{131} in *Torpedo* and *Scyliorhinus* and has found higher values when the animals are approaching spawning. Treatment with goitrogens does not result in any histological change in the thyroid although autoradiographs

showed that there is a blockage of the fixation of protein-bound iodine (yet another indication that epithelial cell height is not always a measure of thyroid activity!). Nor does there appear to be any change in the gonad histology after goitrogen treatment of *Scyliorhinus canicula* (Matty, unpublished).

I would now like to mention a little of my own data which I think demonstrate again a relationship between sex and thyroid in fish. Olivereau, Dodd and myself have independently noted that there is a marked (2-300 per cent) difference in the thyroid weight of mature male and female dogfish of the same size. However, it is not possible to demonstrate this phenomenon in most teleosts due to the diffuse nature of the gland. Recently though it has been possible to show that such a difference also occurs in the parrot fish, *Scarus guacamaia*. When the dried glands were weighed it was noted that the glands of the older, that is, the heavier and longer, male fish weigh less than those of the female (Fig. 4). This difference does not appear to exist between the smaller immature fish.

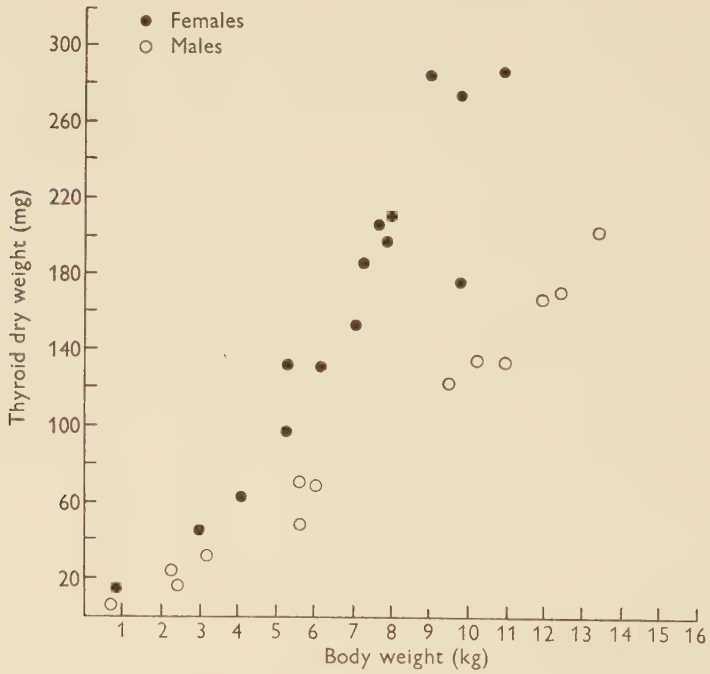


Fig. 4.—Dry weights of thyroids in male and female *Scarus guacamaia*. (Reprinted with permission from *J. Endocrinology* Vol. 19 (1959)).

An analysis of the protein-bound iodine, i.e. hormonal iodine, content has not previously been made for a teleost thyroid, but again working with the thyroid of *Scarus* it has been possible to do this and to demonstrate sex differences (Matty, 1959). The average dry thyroid protein-bound iodine values for fifty-three fish was 0.0205 per cent, but this means little as the range varied from 0·001 to 0·105 per cent. In spite of this variability the percentage

protein-bound iodine does increase with the size and consequently the age of the fish. In males it is generally lower than in females of comparable weight and length, particularly in the 50 to 80 centimetre length range, but again there is some variability. From this and from the fact that there is a sex difference in thyroid weight it follows that the total thyroidal protein-bound iodine values show this sex difference. Here again there is variability, but female thyroids normally contain much more than males of comparable size.

It has not been possible to demonstrate any relationship between the plasma protein-bound iodine with sex or age nor with thyroidal hormone content. There was one more feature of this study which showed a thyroid-reproduction and size relationship, namely that the increase of total thyroidal protein-bound iodine occurs at a more rapid rate than the increase in body weight or length. This means that the amount per gram body weight increases with age, with females appearing to contain a greater amount. This is shown in Fig. 5.

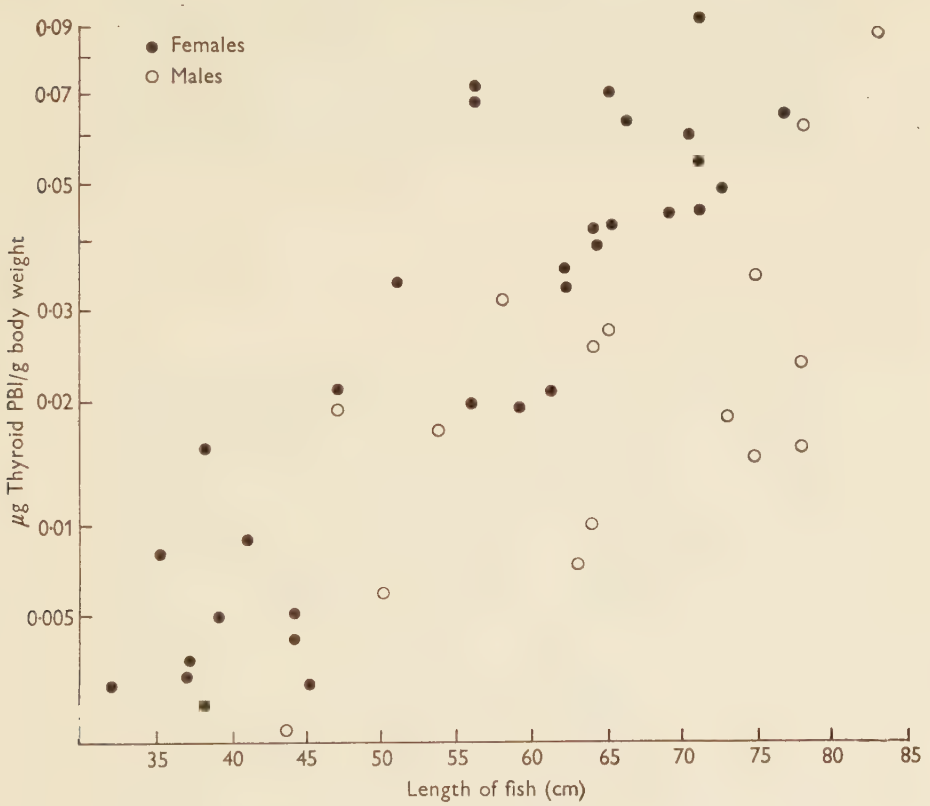
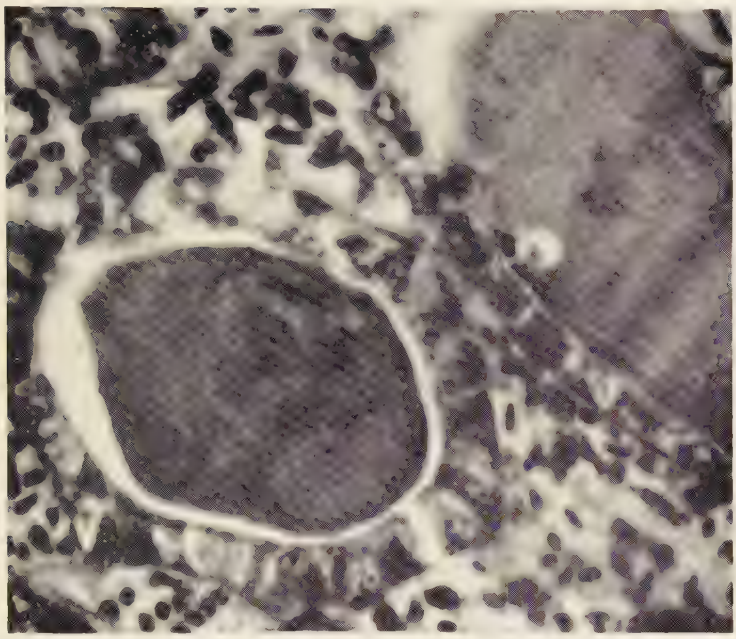


Fig. 5.—Increase in thyroid PBI/g body weight with age (length) in *Scarus guacamaia*. (Reprinted with permission from *J. Endocrinology* Vol. 19 (1959)).

Another recent observation which is, I think, of interest in demonstrating a relationship between thyroid and sex is the effect of androgens on the thyroid of the teleost fish *Sparisoma squalidum*. If this fish is given a single injection of 500μ testosterone phenylacetate or tri-weekly injections of 1·0 mg. methyl

testosterone then within fourteen days the thyroid gland is completely hypertrophied. The epithelium is several hundred per cent higher, and is full of large colloid droplets. The colloid of the lumen has often disappeared and there is marked increase in vascularisation (Pl. 1). That this is a direct effect of the androgen upon the thyroid, and is not a result of stimulation by thyrotrophin, appears to be confirmed by a careful examination of the basophils of the pituitary where no change indicative of thyrotroph hyperfunction could be seen. Teleost thyroids give a histological response to mammalian thyrotrophin, goitrogens and androgens, these treatments possibly helping towards an understanding of natural activity, but the thyroid of cyclostomes and elasmobranchs appear to be quite refactory when treated with thyroid hormone, thyrotrophin or anti-thyroid drugs. I have already mentioned that goitrogens have no effect on the follicle epithelium of elasmobranchs, and also the response to mammalian thyrotrophin is very slight. There does appear, however, to be some pituitary regulation of the thyroid in elasmobranchs, but at an extremely low level (Pickford & Atz, 1957), providing a basis for thyroid variability. In recent preliminary studies on *Myxine glutinosa* it has not been possible to demonstrate any marked histological change in the thyroid after treatment with mammalian thyrotrophin or with thiouracil. A search for a histologically evident thyroid cycle in this cyclostome may be then a vain hope.

Thyroid cycles are probably always influenced by the pituitary in adult teleosts for it is known that a thyrotrophic hormone is secreted by the pituitary, and that mammalian thyrotrophin will also stimulate the thyroid (Pickford, & Atz, 1957). The situation, however, is far from clear in the cyclostomes and elasmobranchs. Whereas the teleosts, *Astyanax* (Atz, 1954), *Phoxinus* (Barrington & Matty, 1955) and *Rutilus* (Matty & Matty, 1959) all appear to possess specific thyrotrophin-producing cells in the pituitary, i.e. thyrotrophs, these have not been demonstrated for cyclostomes or elasmobranchs. In the viviparous cyprinodont *Lebistes reticulatus*, Stolk (1954) correlated an increase in the volume of the adenohypophysis during the latter part of the gestation period with an increase in thyroid activity, but a seasonal change or any other indication of periodicity does not so far appear to have been observed in the thyrotrophs, an investigation that would probably be worth making. Woodhead & Fontaine (1959) have, however, assayed cod pituitary extracts using the uptake of I^{131} by the rainbow trout thyroid as the criterion of potency of thyrotrophin. They did not, though, observe much significant change in the thyrotrophin content of the pituitaries collected in June compared with those collected in October, December or January when the gonads of the cod were mature, in spite of there being histological differences in the thyroid epithelium height between the June and winter fish. There was, however, some indication of a lower hormone titre in the pituitary during the reproductive period which may have indicated an increased output of the hormone during this period. A dissimilar result was obtained by Zaitzev (1955) who assayed the metamorphic potency of the mesoadeno-hypophysis of *Esox lucius* towards anuran tadpoles. According to Pickford it was stated that the thyrotrophic potency was maximal at the time of spawning and that this was correlated with increased activity of the thyroid gland.



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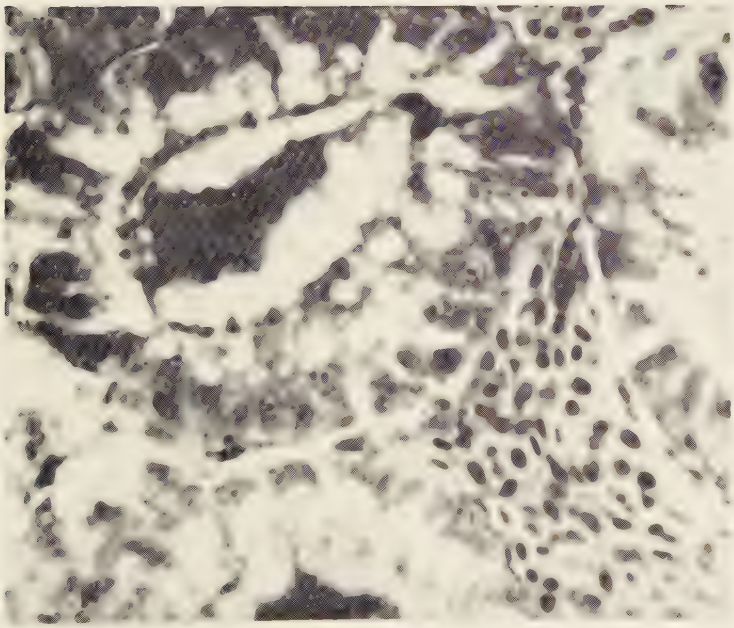

B

A. Normal thyroid of *Sparisoma squalidum*.

B. Thyroid of *Sparisoma squalidum* after treatment with testosterone phenylacetate.

THE PROBLEM OF THYROID FUNCTION

Cycles and regular changes in the " activity " of the thyroid gland of fish, even though the assessments may not in fact demonstrate a change in hormone output, certainly are quite common. An effort must be made now to relate these changes to the possible functions of the gland. No attempt will be made to try to unravel the details of how the pituitary might control these changes as this is discussed elsewhere in this symposium (see Barrington).

Thyroid cyclical activity is not restricted to fish but has been observed throughout the vertebrates and documentation has been made for the amphibia and reptiles by Lynn & Wachowski (1951) and for birds and mammals by Fuhrman (1946), Maqsood (1952) and Pitt-Rivers & Tata (1959). Thermoregulation, hibernation and reproduction have all been related to thyroid activity in these higher vertebrates. Seasonal changes of a calorigenic and thermoregulatory nature are not likely to be influenced by changes in thyroid activity in fish for it has been difficult or impossible to experimentally induce by thyroid treatment or ablation any oxidative metabolic change in fish (Matty, 1954, 1957 ; Hoar, 1958). As the thyroid does not always influence the hibernation cycle in mammals (Uiberall, 1934) it would be idle to speculate on this function of the thyroid gland in fish particularly as this phenomenon is mainly restricted to dipnoans, a group about whose thyroid we know little. Leloup (1958), however, has recently made a study of the thyroid of *Protopterus annectens* and has found that the activity of the gland is considerably diminished during hibernation. Little I^{131} was taken up by the thyroid of the encysted fish, and there was a marked reduction of plasma protein-bound I^{127}. It would seem that this inactivation of the thyroid is a result of a lowered metabolism and there is no evidence that the thyroid initiates hibernation.

Phases of reproduction have been shown to be influenced by the thyroid in amphibia, birds and mammals. The question that must be asked is, is this so in fish or are the cycles that we see in the thyroid merely a reflection of gonadial activity? Is then the cause the sex hormone and the effect, direct or indirect, the increased follicular cell height, and if this is so, to what is the increased hormone output directed? In mammals the difficulty of disentangling the thyroidal function from the many interacting factors such as illumination, temperature, sex and age of animal and stage of sexual development has prevented any generalization, and so it is in fish.

Evidence that the initial stimulus for thyroid activity might arise from the gonads is given in the experiments that have already been described on *Sparisoma squalidum*. There is little evidence that the thyroid directly influences gametogenesis in fish except for the rather doubtful work that has been performed using goitrogens. Indeed, in the minnow *Phoxinus phoxinus*, repeated attempts under many conditions have been made to endeavour to demonstrate a gametogenic effect with thyroid powder, thyroxine and triiodothyronine but without success. This negative result does not mean, of course, that the thyroid could not influence any of the reproductive phases if some other variable was changed, as it might be under natural conditions. A number of Russian workers have observed increased egg production in fish when thyroid is given with pituitary extract over treatment with pituitary

extract alone. Although a direct gametogenic effect of the thyroid hormones has not been shown in fish, the increased thyroxine content of *Fundulus* at spawning observed by Berg and her co-workers must surely indicate an influence of the gland on some part of the fish at this time. Moreover it has been shown in *Phoxinus* that thyroxine will promote the formation of certain secondary sexual characteristics (see Barrington, this symposium). Viewing these heightened activities and experimental studies one must conclude that the thyroid normally plays some synergistic, auxiliary or complementary role, particularly in the female, at many different periods in a fish's life, not only at periods of reproduction and sexual maturity, but also during growth and metamorphosis. The profound, if somewhat erratic, influence of thyroid powder and thyroxine on the growth and metamorphosis of fish has been reviewed by Pickford & Atz (1957), and this aspect is further discussed by Barrington in this symposium.

A non-gametogenic reproductive role and possible involvement in growth and metamorphosis points to some metabolic controlling function but as mentioned previously oxidative metabolism of fish does not seem to be influenced by the thyroid. However, recent findings of Hoar (1958) have shown that thyroxine will produce a marked increase in the excretion of nitrogen from the goldfish as measured by changes in the ammonia of the ambient water, and similar indications are appearing in work now in progress at Nottingham. Also there are many reports in the literature of thyroid powder or thyroxine causing silvering in fish, i.e. the laying down of guanine crystals. Although, as Keilin (1959) has very recently pointed out, the origin of intracellular guanine crystals has not been investigated in fish, guanine is known to be liberated as one of the natural degradation products of nucleic acid. This being so, it seems possible to think of the thyroid hormones in fish being most concerned with protein and nucleoprotein metabolism. Protein-anabolic as well as protein-catabolic effects have been demonstrated both in poikilotherms and homoiotherms treated with thyroid preparations, and fish appear to be no exception. This view of thyroid function does not only account for the activity shown by the gland during the growth and metamorphosis of fish, but also may account for the cyclical occurrence of active glands appearing during the gestation period of *Lebistes* and other live-bearing fish and during periods of rapid gonad maturation. Thus at any period in which vigorous protein anabolism (or catabolism) is occurring, then one might expect to see heightened thyroid activity or increased thyroidal hormone output.

The biochemical mechanisms involved in the effect of thyroid on nitrogen metabolism, as in mammals, are, of course, unknown. Furthermore, one must be constantly aware, as in all other aspects of fish endocrinology, of basing a generalization on one species of fish such as the goldfish. Indeed preliminary experiments at Nottingham on the stickleback and the minnow suggest that their pattern of nitrogen metabolism response after thyroid treatment may not be the same as that of the goldfish. More research is obviously needed.

Although I have emphasised the possible function of the thyroid in protein metabolism and its undoubted effects on the integument and upon maturation and growth, there are two other functions in mammals that may also be impor-

tant in fish, namely the effects of thyroid hormones on the central nervous system and upon lipid metabolism. Gorbman (1959) has suggested that the action of the thyroid on the central and peripheral nervous system is fundamental. If this is so, then the cyclical periods of activity of the gland might be correlated with periods of increased movement and other behavioural changes resulting from nervous stimulation. In addition to the evidence tabulated by Gorbman it has been shown by Fontaine & Leloup (1952) that blood protein-bound iodine values are much increased at the time of salmon migratory movements. Leloup (1949c) has made the suggestion that high levels of blood protein-bound iodine are associated with active fish and low levels with sluggish fish. However, recent observations (Matty, 1959) have shown that an extremely active marine fish *Scarus guacamaia*, may have protein-bound iodine values of a comparatively low level. Fontaine & Leloup (1959) have observed that the muscle iodine of the rainbow trout is depleted after being made to swim against a current of water. By measuring I^{131} uptake, plasma I^{131} and plasma protein-bound I^{127} they were able to show that the thyroid gland became hyperactive. They suggest that the swimming against the current depletes the muscle of iodine which is replaced from the plasma which in turn is replaced from the thyroid. Thus motor activity hastens thyroidal activity. It is possible then that the thyroid, via the hypophysial system, might initially provide the stimulus for a migratory movement, but that once initiated, thyroid activity becomes more concerned with replacement of plasma iodine utilized in muscular activity. Could it be that this concern is with some aspect of muscle protein metabolism?

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CYCLICAL ACTIVITY OF THE THRYOID GLAND OF FISH IN RELATION TO ENVIRONMENTAL CHANGES

BY

D. R. SWIFT

Freshwater Biological Association, Ambleside, Westmorland

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INTRODUCTION

It is an established fact that in many fish the activity of the thyroid gland varies in a cyclical fashion and that this cycle of activity varies with the solar seasons ; this being so then the activity of the gland must vary either as a result of a change in the animal's external environment, or else this cycle of activity must be an inherent one. That the rhythm could be inherent is not as impossible as it may appear at first sight, for many different types of animals have been shown to display inherent rhythms, and Brown (1946) has shown some evidence for this in the growth rate of brown trout living in constant environments. If the growth of these animals varies with an inherent rhythm it is not perhaps so impossible to suppose that the activity of the thyroid may also do so. However, it seems significant that such inherent rhythms as have been demonstrated bear a relationship to the changes of the solar seasons and would therefore seem to have been impressed on the animals from the external environment. Assuming, therefore, that the factor which changes the level of activity of the fish thyroid is some external environmental factor which varies in a seasonal manner, then it will be extremely useful for us to know what this factor is ; we still cannot say for certain what is the function of the thyroid in fish, and such information could conceivably give us some valuable clues. When we try to determine which external environmental factor will, when it changes in value, provoke a change in the animal's internal environment, we are up against a most difficult problem. Obviously we are interested in the chain of events as it occurs in the animal in nature, and equally obviously as soon as we place a fish in an experimental aquarium it is not in its natural surroundings. Here, of course, is one of the pitfalls of experimental zoology and one which ecologists are forever pointing out, namely that the animal and its environment are inseparable. I do not think that physiologists are as guilty of forgetting this

as some people think, but it is a vitally important point to bear in mind when thinking about the possible causes of an endocrine cycle induced in an animal by changes in its external environment, and in particular when performing experiments with the animal in which it is subject to an artificial environment.

METHODS OF MEASURING THE ACTIVITY OF THE THYROID

As Matty (this symposium) has explained, the methods available for the study of the thyroid fall into two groups. The histological method, depending on the measurement of variations in the heights of the epithelial cells of the thyroid as found at different times of the year and after various experimental procedures, is claimed to give a true reflection of the activity of the gland, but as I hope to show later it is possible to have a change in glandular activity without a corresponding change in the height of these cells. Before radio-iodine was available this histological investigation was the only method possible, and recent workers who have used it include Barrington & Matty (1954), Woodhead (1959), Fortune (1955), Olivereau (1955), and Swift (1959), to mention only a few.

The second method of measuring the thyroid activity involves the use of radio-iodine. This has been used in three ways, either by measuring the uptake of iodine or the loss of iodine by the gland, or the amount of labelled hormone produced by the gland. The accumulation of iodine by the gland has been measured by Robertson (1958) and by Gorbman & Berg (1954), using direct counting techniques, and by Olivereau (1955), using the degree of blackening of auto-radiographs. Berg and her colleagues (1959) have also measured the amount of labelled hormone produced by the gland, while Swift (1959) has measured the rate of loss of iodine from the gland as an index of its activity.

Of these methods the measurement of the amount of labelled hormone produced is the most accurate way of assessing the activity of the gland, provided that we remember that we are only assessing the activity in terms of the particular hormone measured. So we have four ways of measuring the activity of the gland and we do not fully understand the relationship between the results obtained by these four different methods. Due to the work of Berg, Gorbman, Leloup and Fontaine we know something about the types of hormone produced in the fish thyroid but we do not know whether the ratios in which these hormones are produced varies seasonally, what a change in the epithelial cell height means in terms of output of hormones, or what the relationship between iodine uptake and hormone output is, and until we understand these relationships we cannot compare the results obtained by the different methods. To understand these relationships more than one variable must be measured, and there are three cases of two methods being used at the same time. Olivereau (1955) has measured the epithelial cell height and the iodine uptake, Berg *et al.* (1959) have measured the iodine uptake and radio-thyroxine production, and Swift (1959) has measured the epithelial cell height and the rate of loss of radio-iodine. These results will be referred to when the effect of temperature and light on the thyroid is considered.

EFFECT OF SOLUTE CONCENTRATION

In the environment in which the fish lives there are only four factors which vary seasonally, the water temperature, the length of the photo-period in each day, the amount and kind of solutes in the water, and the available food supply. The variations in the animal food supply are seasonal, but are so small as to be probably insignificant in initiating any change in the animal's physiology.

A change in the concentration of solutes in the environment can affect the thyroid in two ways, either directly by a change in the level of available iodine, or indirectly as a result of the change in the osmoregulation of the fish.

Unfortunately we know very little about what changes, if any, occur in the level of iodine in bodies of water at different times of the year. It is a fact that the level of iodine in the water influences the activity of the thyroid. In the extreme case goitrous conditions can result (Marine, 1914), and in normal environments Gorbman & Berg (1959) have shown that the thyroid responds to a change in iodine level. However on the evidence as yet available it is not easy to distinguish between this effect and a possible response by the thyroid to a change in osmoregulation. That the thyroid is implicated in osmoregulation seems evident, but the evidence as to the part it plays is confused. Koch & Heuts (1942) found that oral administration of thyroid hormones to sticklebacks living in sea water caused a fall in their osmoregulatory faculties which resulted in an increase in the osmotic pressure of the blood. This change in the fishes' osmoregulation Koch & Heuts supposed to be connected with the annual migration of sticklebacks to and from fresh water. This work is supported by the results of Baggerman (1959) with these animals. Baggerman showed that in nature there is a close relationship between the gonad cycle and the salinity preference of these animals, they preferring freshwater during the spring breeding season which is also the time when the thyroid gland of the fishes is active. By means of gonadectomy and thyroxine and thiourea treatment Baggerman demonstrated that it was the high thyroid activity and not the mature gonads which in all probability caused the change in the animals' osmoregulation. Hickman, as reported by Hoar (1959), finds that in the flounder also the thyroid is implicated in the osmoregulatory mechanism. However, he finds that, contrary to the work with the stickleback, the flounder's thyroid is more active in the sea than in fresh water. This conclusion he reaches after adjusting the iodine level to the same value in both waters. As Hoar (1959) points out, some cases of thyroid hyperplasia may be due to a lack of iodine in the environment and not, as has been suggested in the case of several species of anadromous fish in fresh water, to an increased need of thyroid hormones. That such a mistaken interpretation could possibly occur serves to emphasise the importance of estimating the activity of the thyroid in terms of output of hormones, and not in histological appearance.

The connection between the activity of the thyroid and the concentration of solutes in the environment, although an extremely interesting one, is not strictly relevant to this discussion for the influence cannot be instrumental in causing the regular annual cycle of thyroid activity since the changes in the solute concentration are of two types, either a daily rhythm, as experienced by an estuarine fish, or else a change such as is experienced by a migratory

fish. The first type, although it may influence the thyroid, cannot be the cause of an annual cycle and the second type of change occurs at a special time in the fish's life, and therefore cannot be concerned in the regular annual cycle of activity of the thyroid. Furthermore, in the case of the salmon the increase in thyroid activity occurs before the fish moves into a different environment. So it cannot be that the change in environment is the primary cause of the change in thyroid activity ; it may affect it, but it does not initiate it. It is important to differentiate between changes which are contemporary with changes in the thyroid activity, but do not affect it, and those changes which affect the thyroid. As the thyroid gland has a basic annual cycle of activity then other contemporary changes are associated with this cycle, and it is difficult to disconnect thyroid activity with the many things with which it has been supposed to be associated. So it would appear that, as Hoar (1959) has already pointed out, in these latitudes there are only two external environmental factors which vary in the same rhythmical fashion each year ; these are the length of the photoperiod each day and the water temperature. Before one can consider the effect of the changes in these two factors it is necessary to establish the form of the seasonal cycle of activity of the thyroid in fishes. To do this one must compare the results of different workers and here the difficulties of interpreting the activity of the gland in terms of the different criteria used becomes apparent.

THE ANNUAL CYCLE OF ACTIVITY

Figure 1 shows the the results of Barrington & Matty (1954), Woodhead (1959) and Swift (1959) for the seasonal variation in the height of the thyroid epithelial cells of three different fishes. As can be seen there is a remarkable similarity between these curves for these three different fishes. In each case the epithelial cell heights indicate a high glandular activity during winter and spring and a low activity during the summer.

Figure 2 shows the seasonal changes in the activity of the gland of *Fundulus heteroclitus* as measured by Berg and her colleagues using the iodine uptake and the production of labelled thyroxine by the thyroid as the criteria of the activity of the gland.

The seasonal variation in iodine uptake by the thyroid in this fish varies in a similar fashion to the epithelial cell heights shown in Fig. 1, but the production of radio-thyroxine shows a completely different picture, emphasizing the difficulties of correlating results obtained by different methods.

Figure 3 shows the annual cycle of activity of the thyroid of immature brown trout (Swift 1959) measured by the rate of loss of radio-iodine from the gland. This curve shows two peaks of activity, one in spring, which corresponds to the peak reflected in the increased epithelial cell height (Fig.1), and a second peak in midsummer, which does not correspond with any increase in epithelial cell height. This is further evidence of the dangers of measuring the activity of the gland solely in terms of one criterion.

Olivereau (1955) found that in *Salmo gairdnerii* glands with high epithelial cells picked up more radio-iodine than did glands with low epithelial cells, as indicated by the degree of blackening of auto-radiographs of sections of the gland.

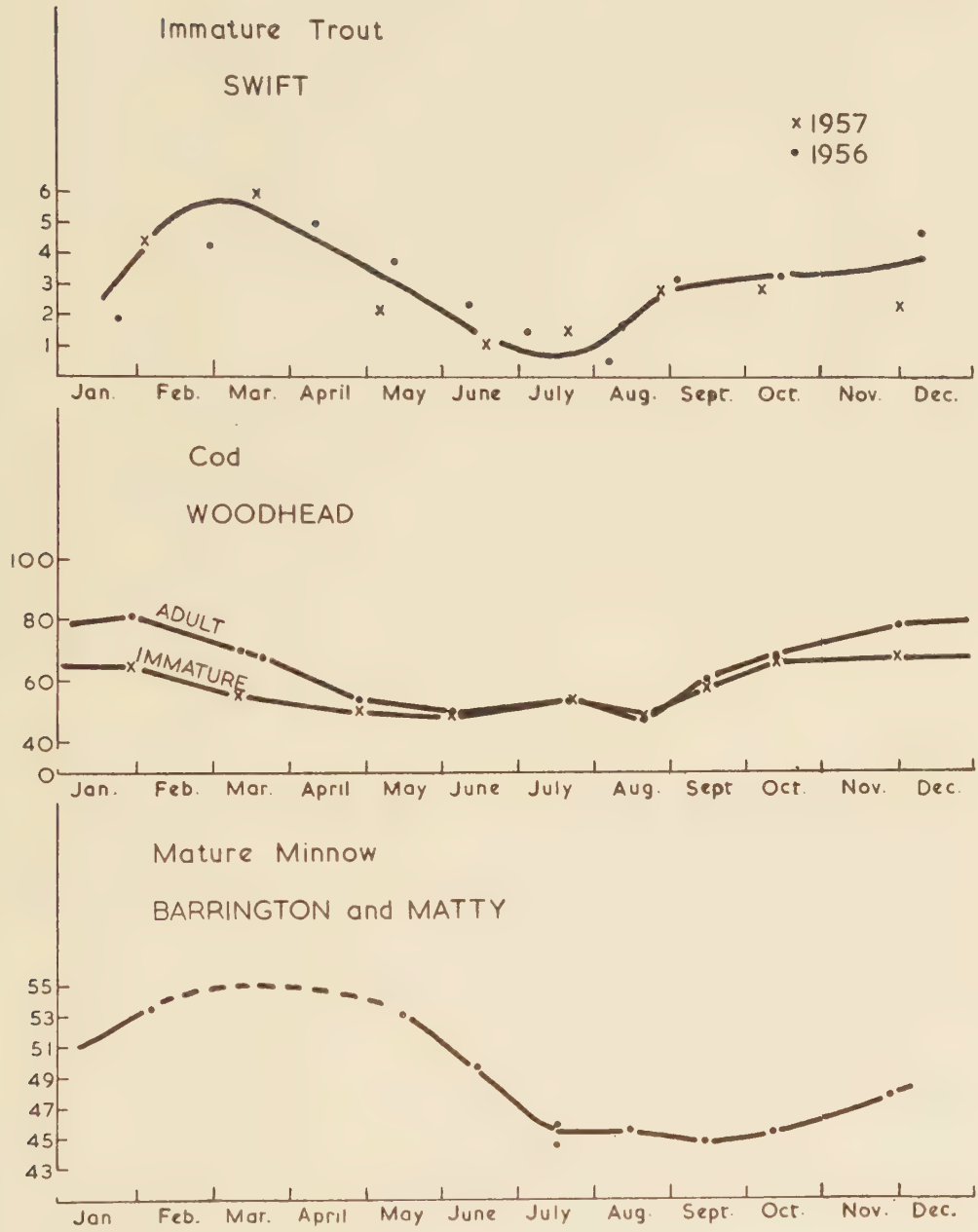


Fig. 1.—Seasonal variation in the thyroid epithelial cell heights of the cod, minnow and trout. Taken from Woodhead (*J. Mar. biol. Ass. U.K.* Vol. 38, 1959), Barrington & Matty (*Proc. zool. Soc. Lond.* Vol. 124, 1954) and Swift *J. exp. Biol.* Vol. 36, 1959). The heights of the trout cells are in microns, those of the cod and minnow in eyepiece units.

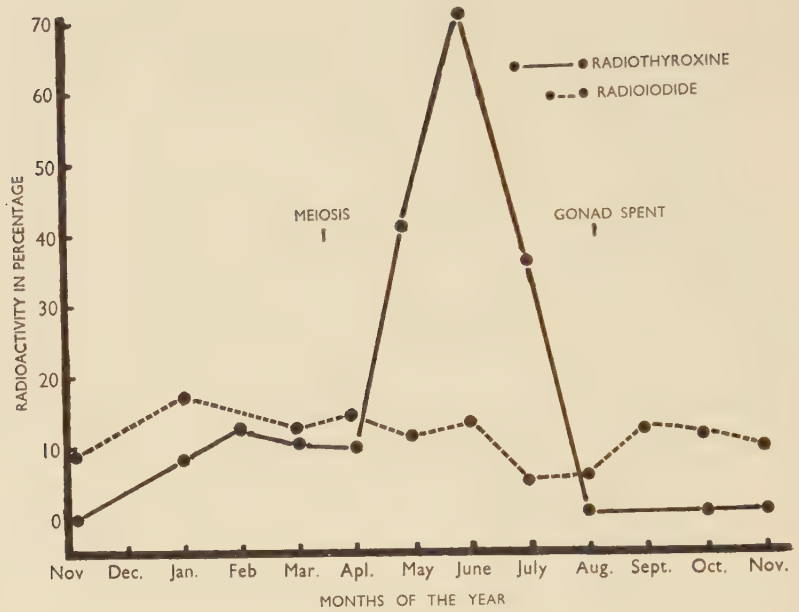
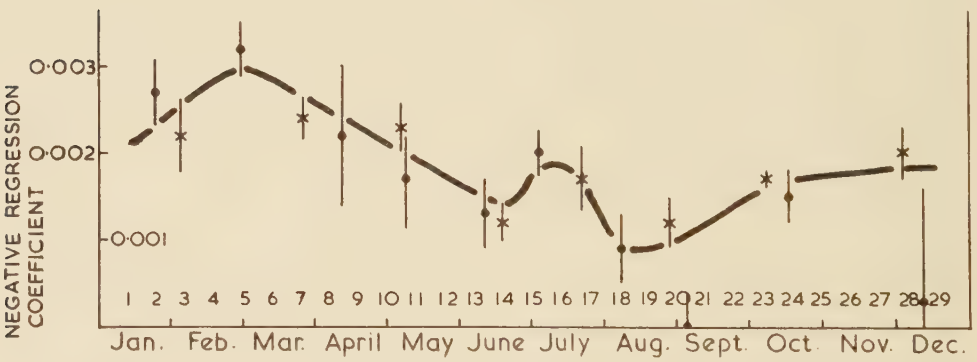


Fig. 2.—The seasonal variations in radioiodine uptake and labelled thyroxine production in *Fundulus heteroclitus*. (Redrawn from Gorbman, *Comparative endocrinology*, 1959, John Wiley & Sons Inc.


NEGATIVE REGRESSION COEFFICIENT
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0·002
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Jan. Feb. Mar. April May June July Aug. Sept. Oct. Nov. Dec.

Fig. 3. Seasonal variation in the rate of loss of radioiodine from the thyroid gland of yearling brown trout. (Reprinted from Swift, *J. exp. Biol.* Vol. 36, 1959).

It would appear that the annual cycle of thyroid activity in many species of fish living in our latitudes follows a similar pattern with a maximum activity during the winter and spring and minimum activity during the summer, periods of high activity being indicated by an increase in the epithelial cell height of the gland, an increased uptake of radio-iodine, and an increased rate of loss of radio-iodine. Superimposed on this cycle in mature fish there is apparently another one. Here high activity is accompanied by an increase in the production of thyroxine in *Fundulus heteroclitus* (Fig. 2). In the brown trout the

difference in the activity of the glands of mature and immature fish is very apparent (Swift 1954, 1959). In the mature fish the maximum rate of loss of iodine from the gland occurs at the time of the ripening of the gonad, while in the immature fish the maximum is in spring. Elsewhere in this symposium Matty has considered the strong evidence suggesting that the thyroid is concerned with gonad maturation. It would appear that this second period of high activity in the glands of mature fish is also concerned with gonad maturation. From the results on *Fundulus* it is reasonable to suppose that at these times the thyroid produces more thyroxine than at other times. Perhaps the different hormones produced by the thyroid have different target organs.

As different fish spawn at different times of the year, then the increased activity of the thyroid at these times is yet another hazard in the comparison of thyroid activity cycles in different fishes.

The basic annual cycle occurs in immature and mature fish alike and seems to be similar in fish as different as the trout, the minnow, the cod, and *Fundulus heteroclitus*. This cycle takes the form of high activity during the winter and spring and low activity during the summer. Periods of high activity are reflected by an increase in epithelial cell height, iodine uptake, and the rate of iodine loss from the glands. We do not know what function the thyroid performs by varying in activity in this manner, and it is with the possible causes of this cycle that I wish to deal now.

EFFECT OF LIGHT AND TEMPERATURE

As I said previously there are two possible causes for such a constant annual cycle, these being varying photoperiod length and varying water temperature. To see if there is any obvious relationship between the thyroid activity in the brown trout and these two factors I have taken the activity as measured by the rate of loss of radio-iodine from the gland and plotted it against photoperiod length and water temperature.

Figure 4 shows the thyroid activity plotted against the length of the photoperiod. It seems to me that there is no obvious relationship between the two, and that any apparent relationship is merely a reflection of the fact that in these latitudes the length of the photoperiod and water temperature vary together over the year in a very similar fashion.

Figure 5 shows thyroid activity plotted against the water temperature and shows I think good evidence that the thyroid activity is inversely proportional to the water temperature, with the exception of the summer activity peak, which as I have said I believe is concerned with the gonads. However, I think that the thyroid is not solely influenced by the changing water temperature, for during the winter when the water temperature is falling the slope of the line is not the same as it was during the period of rising temperatures in spring. Over this time of the year the points of equal photoperiod length are joined together and it seems that here the gland is responding both to a falling water temperature and a changing photoperiod length. Over all this period the thyroid is increasing in activity as the water temperature falls, but over the first half

of this period it is depressed in activity by the shortening photoperiod and then, as the days begin to lengthen, the activity of the gland increases.

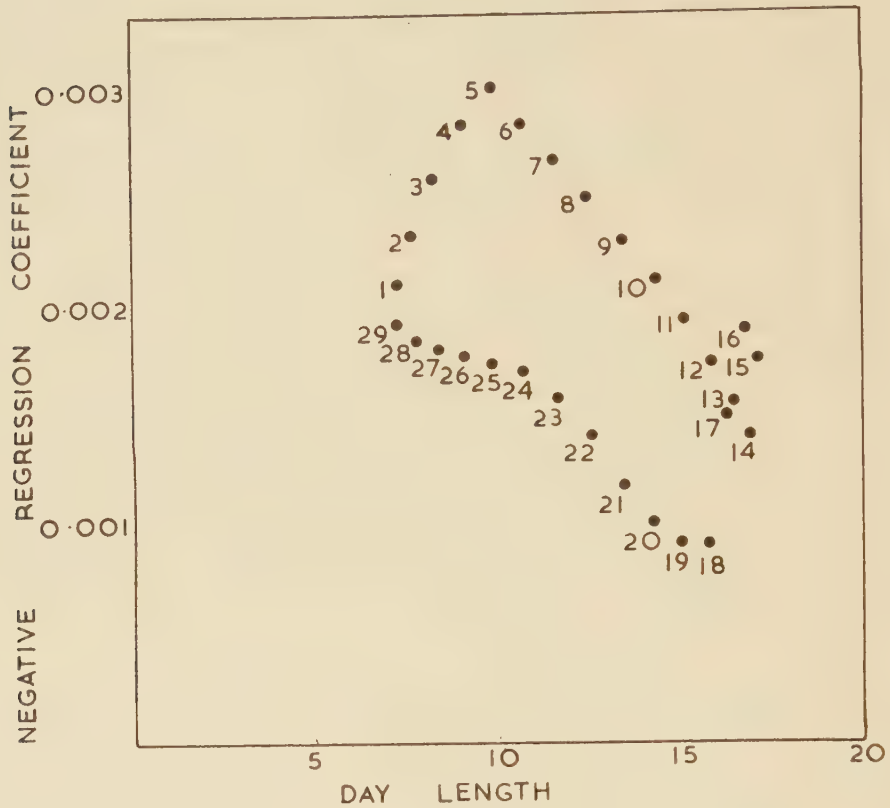

Fig. 4.—Thyroid activity in yearling brown trout taken from Fig. 3 plotted against the average length of the daily photoperiod. The numbers by the points are the same as those in Fig. 3 and indicate the time of year.

This I believe is the cause of the basic cycle of the activity of the thryoid. It remains to be seen, however, what function the gland is serving by altering its activity in this manner. I have suggested that it is acting as a temperature compensating mechanism (Swift 1959). There is little clear experimental evidence of the effect of temperature and photoperiod length on the activity of the thyroid. Barrington & Matty (1954) report that the thyroid epithelial cells of the minnow increased in height with increasing water temperature. However their fish were kept in total darkness. Olivereau (1955 a and b) as previously stated, found that in *Salmo gairdnerii* the epithelium increased in height in cold water and decreased in warm; however, she failed to find any response to temperature in the glands of the carp, the tench, the eel, the chubb and the dogfish. Woodhead (1959) points out that there is no obvious relationship between the epithelial cell height in the cod thyroid and the water temperature. It may be however that the cod lives in waters too cold for

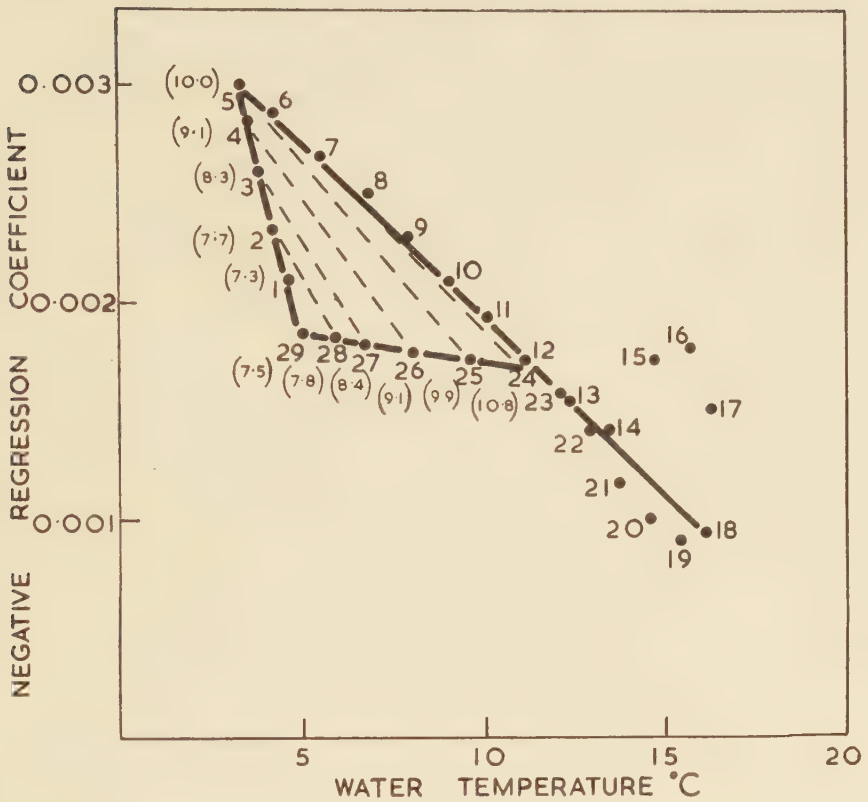

Fig. 5.—Thyroid activity in yearling brown trout taken from Fig. 3 plotted against the water temperature. The numbers by the points are the same as in Fig. 3 and indicate the time of year. Points of equal photoperiod length, given by the figures in brackets, are joined by dotted lines.

this effect to be obvious and where the depressing effect of the low temperature on the rate of enzymic reaction in the fish is dominant. Berg *et al.* (1959) report that in *Umbra limi* more labelled triiodothyronine is produced in cold water than in warm.

On the whole the evidence supports the idea that in nature the thyroid of the fish responds in a normal fashion to a combination of both changing photoperiod length and temperature. This is after all what a fish living in these latitudes normally experiences, so it may be a mistake to expect a normal response from the gland if the fish is held under constant environmental conditions.

CONCLUSION

There appear to be two types of activity of the thyroid gland of fish living in our latitudes. One type is connected with gonad maturation and results in an increased activity of the gland at spawning time. This activity is reflected by an increase in the rate of loss of radio-iodine (*Salmo trutta*), and a great increase in the production of labelled thyroxine (*Fundulus heteroclitus*).

The second type of activity of the thyroid occurs in a regular annual cycle in both mature and immature fish. In this type periods of high activity are indicated by an increase in the epithelial cell height, an increase in the uptake of radio-iodine, and an increase in the loss of radio-iodine. It is suggested that this cycle of activity results from the influence of the changing water temperature and the changing length of the daily photoperiod, temperature being the major controlling factor. The function of the thyroid in this cycle is not known but it has been suggested (Swift 1959) that it acts as a temperature compensating mechanism. There is as yet however no conclusive experimental evidence as to the cause and function of this cycle of activity.

In conclusion, it is suggested that future profitable research would be to try to establish the amounts of the different hormones produced by the thyroid at different times of the year in both mature and immature fish from species spawning at different times ; also we must try to establish experimentally the reaction of the thyroid to varying day lengths and water temperatures, bearing in mind that in nature the animal never experiences constant environmental conditions.

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THE INFLUENCE OF INTERNAL AND EXTERNAL FACTORS IN THE REGULATION OF THE SPERMATOGENETIC CYCLE IN AMPHIBIA

BY

P. G. W. J. VAN OORDT

Zoological Laboratory, Dept. of Endocrinology, Utrecht, The Netherlands

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INTRODUCTION

When, in the second half of the seventeenth century, the Dutch scientist Jan Swammerdam gave an accurate description of the sex organs and the mating behaviour in the frog (cf. Schierbeek, 1946), the first step was taken towards the investigation of the sexual cycle in Amphibia.

The study of that part of the male sexual cycle known as the spermatogenetic cycle had to wait for the development of good microscopes and histological techniques and, therefore, was not studied before the second half of the nineteenth century. Since that time, however, the yearly formation of sperm cells and their evacuation from the testis tubules have been studied in many species of Amphibia, living in cold, temperate and tropical regions.

The experimental approach to problems concerning the regulation of the spermatogenetic cycle by internal and external factors dates from the year 1923, when Giusti & Houssay found that hypophysectomy leads to atrophy of the testes of *Bufo arenarum*, and from 1924, when Witschi studied the influence of climatic conditions upon spermatogenesis and breeding in *Rana temporaria*. Since those days many contributions have been made to our knowledge of the physiology of the spermatogenetic cycle.

It is the purpose of this review to present a short survey of these contributions, particularly those dealing with the regulation of spermatogenesis. The reader

is also referred to the reviews by Galgano & Mazzi (1951), Galgano (1952), Houssay (1954), G. J. van Oordt *et al.* (1955, 1959), P. G. W. J. van Oordt (1956a) and Gallien (1959).

THE SPERMATOGENETIC CYCLE

It is clear from each of the numerous publications dealing with the amphibian spermatogenetic cycle that this cycle mainly consists of two processes, namely the formation of sperm cells, or spermatogenesis, and the release of these cells from the testis tubules or ampullae. For the latter process G. J. van Oordt & Klomp (1946) introduced the name " spermiation ".

Spermatogenesis begins in all Amphibia with multiplication of primary spermatogonia or protogonia, which are situated against the wall or basal membrane of the testis tubule or ampulla. Each of the primary spermatogonia is surrounded by flattened follicle or cyst cells. By the division of a primary spermatogonium either new primary spermatogonia or secondary spermatogonia are formed. The latter occurs when the daughter cells remain together, surrounded by the original follicle cells, and form a cell nest. New primary spermatogonia may be formed, however, if the daughter cells separate, each becoming surrounded by follicle cells. During the multiplication period of spermatogenesis the number of secondary spermatogonia in the cell nests increases.

This period is followed by the growth period during which the secondary spermatogonia increase in size and become primary spermatocytes. During the ensuing maturation period the primary spermatocytes undergo the prereductional stages and divide into secondary spermatocytes, these then dividing into spermatids. The initially spherical spermatids elongate and change into sperm cells. This process is generally called spermatohistogenesis.

In the cell nests containing primary spermatocytes small intercellular vacuoles may be seen. These increase in size and coalesce to form one big central vacoule by the time spermatohistogenesis takes place. The increasing vacuole pushes the spermatids against the wall of the cyst. The cell nest then bursts after which the wall is folded and the follicle cells change into Sertoli cells. These are cup-shaped, each cup surrounding the heads of a bundle of sperm cells. Thus, every cell nest changes into a number of sperm bundles.

In many Amphibia the production of sperm cells takes place throughout the year. Their spermatogenetic cycle is thus a continuous one. To this group belong the Indian species *Rana erytrea*, *Rana grahami* (Champy & Coujard, 1939), *Rana tigrina* and *Bufo melanostictus* (Basu, personal communication), and the South-American species *Bufo arenarum* (Burgos & Mancini, 1948), *Bufo paracnemis*, *Bufo granulosus d'orbignyi*, *Leptodactylus ocellatus reticulata* and *Leptodactylus ocellatus bonaerensis* (Cei, 1949 a, c). However, this group not only contains the above tropical or subtropical species, but also *Telmatobius schreiteri* (Cei, 1949b) and *Hyla raddiana andina* (Caruso, 1949), both living at high altitude in the Andes mountains, where the winter temperatures are very low.

Other Amphibia have a discontinuous spermatogenetic cycle, i.e. spermatogenesis is confined to a certain period of the year. In all species living in

temperate regions spermatogenetic activity is only found during the second half of the spring and during the summer months. During autumn and winter spermatogenesis is either impaired or completely absent. Also in the South-American species *Leptobatrachus asper*, *Leptodactylus bufonius* (Cei, 1949 c) and *Phyllomedusa sauvagii* (Caruso, 1949) spermatogenesis is interrupted during the colder part of the year. In *Leptodactylus ocellatus typica* Cei (1949 a) found two interruptions, one during the cold winter and the other during the very hot summer days.

In two species, *Rana esculenta ridibunda* (Galgano, 1935 b) and *Discoglossus pictus* (Champy, 1923 ; Kehl, 1944), it is known that the individuals living in subtropical mediterranean regions have a continuous spermatogenetic cycle, whereas those living in temperate climatic conditions of Europe have a discontinuous spermatogenetic cycle.

Since an interruption or resting period in the spermatogenetic cycle is found mainly in Amphibia living in those areas where the variations in ambiental circumstances are relatively large, it is likely that spermatogenesis is advanced by one or more external factors.

In addition to these external influences, certainly internal factors also regulate the spermatogenetic processes. The importance of pituitary gonadotrophin, for example, follows from the fact that variations found in the pars distalis of the pituitary correspond to seasonal changes of the germinal epithelium. Such correlations have been found in *Rana temporaria* (Sklower, 1925), *Bufo arenarum* (Masselin ; 1940, Prieto Diaz & Echave Llanos, 1947), *Necturus maculosus* (Aplington, 1942), *Triturus palmatus* (Tuchmann-Duplessis, 1943), *Triturus cristatus carnifex* (Mazzi & Pieri, 1949), *Bufo vulgaris* (Zuber Vogeli, 1953) and *Taricha torosa* (Miller & Robbins, 1955).

Many of the experimental data concerning the influence of pituitary gonadotrophin and of other internal agents, as well as those concerning the effect of external factors upon spermatogenesis, are discussed below.

REGULATION OF THE SPERMATOGENETIC CYCLE BY INTERNAL FACTORS

The influence of the pituitary

Ablation of the pituitary

The influence of an endocrine gland on the development or the function of other parts of the body is usually studied by the extirpation of the hormone-producing organ and by its re-implantation or the injection of its extract or of purified hormones.

These methods have been used in the study of the function of the amphibian pituitary from the very early days of such experiments. Adler in 1914, and Smith and Allen in 1916, studied the effect of hypophysectomy on metamorphosis in frog larvae. In 1923 and 1924 Guisti & Houssay found that in *Bufo arenarum* hypophysectomy leads to atrophy of the testes. In 1929 Houssay & Giusti ; Houssay & Lascano-González ; and Houssay, Giusti & Lascano-González not only confirmed these results, but also found that the same results can be obtained by extirpation of the pars distalis only. In both cases the testicular atrophy appears to be partly due to a reabsorption of

spermatogenetic elements. Hypophysectomy also prevents a compensatory hypertrophy of the testis following subtotal castration (Houssay & Lascano-González, 1935).

The effect of hypophysectomy on the germinal epithelium in *Bufo arenarum* has been studied in greater detail by Burgos (1950). According to this author extirpation of the pars distalis causes first of all disintegration of the spermatids. This is followed by degeneration of the spermatocytes and secondary spermatogonia, but the primary spermatogonia are only deprived of their mitotic capacity. The sperm themselves are no longer arranged in bundles and slowly degenerate, owing to the atrophy of the Sertoli cells.

Impairment of spermatogenetic activity following hypophysectomy was also found by Woronzowa & Blacher (1930) in *Amblystoma spec.*, *Pleurodeles waltlii* and *Triturus cristatus*, by Burns & Buyse (1932) in *Amblystoma tigrinum*, by Bellerby & Hogben (1938) in *Xenopus laevis*, by Rey (1939) in *Bufo vulgaris*, by Sluiter, G. J. van Oordt & Mighorst (1950) in *Rana esculenta* and by Burgos & Ladman (1957) in *Rana pipiens*. Negative results have only been obtained by Derevici, Derevici & Dornescu (1940) following hypophysectomy in *Rana esculenta*.

The effect of hypophysectomy or ablation of the pars distalis in *Rana temporaria* appears to depend upon the time when the experiment is started and ended. Gallien (1938, 1940) found no visible effect of hypophysectomy during the resting period, when only primary spermatogonia and sperm bundles are present in the testis tubules. The primary spermatogonia, however, lose their mitotic capacity, so that no spermatogenetic activity takes place in animals hypophysectomized before the onset of spermatogenesis in spring. We have confirmed Gallien's results (P. G. W. J. van Oordt, 1956 a), but in addition have observed disintegration of sperm cells in hypophysectomized animals kept under laboratory conditions during the resting period.

Gallien (*op. cit.*) and Sluiter, G. J. van Oordt & Grasveld (1950) found disintegration of secondary spermatogonia in common frogs hypophysectomized at the beginning of spermatogenesis. Adenohypophysectomy, carried out during the final stages of spermatogenesis (P. G. W. J. van Oordt, 1956 a), however, clearly showed that the development of spermatocytes and spermatids into sperm cells can take place without gonadotrophic hormones. In the testes of all animals thus treated a normal amount of sperm bundles was formed, the only difference between adenohypophysectomized and control animals being the number of primary spermatogonia, which was increased in the control frogs only.

Injection of pituitary extracts and gonadotrophic hormones

Although the experiments on the effect of hypophysectomy and ablation of the pars distalis of the pituitary gland indicate the presence of a spermatogenesis-inducing hormone in Amphibia, investigations on the effect of administration of amphibian or mammalian pituitaries and mammalian gonadotrophin preparations are necessary for the right understanding of the importance of the pituitary gland in the regulation of the spermatogenetic cycle.

The first experiments of this type were carried out by Houssay and his co-workers. In 1929 Houssay & Lascano-González treated *Bufo arenarum* with toad's pituitary or pars distalis, finding hypertrophy of the testes and stimulation of the spermatogenetic activity. Comparable results were obtained in *Bufo d'orbignyi* and *Leptodactylus ocellatus* by Houssay, Guisti & Lascano-González (1929). These experiments, together with those of Canturisvili (1936) using *Rana esculenta*, of Schreiber & Rugh (1945) and Burgos (1955) using *Rana pipiens*, and of Sluiter, G. J. van Oordt & Grasveld (1950) using *Rana temporaria*, indicate that in the Amphibia spermatogenesis is evoked and accelerated by a gonadotrophic principle produced by the pituitary gland.

That spermatogenesis is not only affected by amphibian but also by mammalian pituitary extract has been shown by Burns & Buyse (1934) in immature *Amblystoma tigrinum*, by Galgano (1914) in adult *Triturus cristatus* and by Kehl (1944) in *Discoglossus pictus*. Comparable effects were obtained by the injection of serum gonadotrophin in many species, e.g. *Bufo arenarum* (Galli-Mainini & Pinto, 1947 ; Burgos & Rufino, 1953), *Rana temporaria* (G. J. van Oordt, Creutzberg & Spronk, 1949 ; Sluiter, G. J. van Oordt & Grasveld, 1950), *Leptodactylus chaquensis* (Andreozzi, 1952 ; Cei & Acosta, 1953) and *Taricha torosa* (Miller & Robbins, 1954). In these species chorionic gonadotrophin has hardly any or no effect on spermatogenesis. This hormone preparation mainly induces the release of sperm cells from the testis tubules, whereas serum gonadotrophin can cause at most only a slight degree of spermiation.

Burgos & Ladman (1957) have found that low doses of purified follicle stimulating hormone (FSH) increase the number of primary spermatogonia in *Rana pipiens*, but induce no spermiation or interstitial cell stimulation. Low doses of purified luteinizing hormone (LH) appear to have an opposite effect. It is, therefore, not unlikely that in Amphibia, as in other vertebrates, the pituitary secretes more than one gonadotrophic hormone. If this is true, one might expect the pituitary to secrete the LH- like factor prior to and during the spermiation period and the FSH-like hormone during the spermatogenetic period. As far as I know, however, there is up to now no experimental proof for the existence of two different gonadotrophic hormones in Amphibia.

Another question concerning the effect of pituitary gonadotrophin upon spermatogenesis in Amphibia is whether the seasonal changes in spermatogenetic activity are caused exclusively by changes in the gonadotrophin secretion by the pituitary, as has been thought by some investigators. Experiments to solve this problem have been carried out in our laboratory using *Rana temporaria* (G. J. van Oordt, Sluiter & P. G. W. J. van Oordt, 1951 ; P. G. W. J. van Oordt, Sluiter, & G. J. van Oordt, 1952). An extract of frogs' pituitaries, collected during the hibernation period, was injected into normal and hypophysectomized frogs during ten days in the resting period, when, besides sperm bundles, only primary spermatogonia are present in the testis tubules. This extract was also injected into normal and hypophysectomized frogs two months later, when the primary spermatogonia have produced the first few small cell nests containing secondary spermatogonia. In the first experiment the pituitary extract caused the production of small cell nests containing secondary spermatogonia. In the second, however, the production of secondary

spermatogonia was much more advanced and, moreover, a considerable number of primary spermatocytes and even some secondary spermatocytes were formed. In each experiment the difference between normal and hypophysectomized frogs was comparatively small. The most feasible explanation of the difference in effect of pituitary extract injections is to assume that during the resting period the sensitivity of the germinal epithelium to pituitary gonadotrophin is less marked than at the beginning of the spermatogenetic period.

In order to confirm this hypothesis an extract of frogs' pituitaries, collected in summer at the height of the spermatogenetic activity, and a similar extract of pituitaries, collected during the resting period, were injected into hypophysectomized frogs during ten days in January. The testis tubules of control frogs contained, besides sperm bundles, primary spermatogonia and an occasional secondary spermatogonium. In the testes of the frogs, injected with summer pituitary extract, as well as in the testes of those injected with winter pituitary extract, the number of secondary spermatogonia was increased and even a few primary spermatocytes were found. The summer pituitary extract had been slightly more effective than the winter extract, but this effect was distinctly smaller than that obtained with winter pituitary extract at the beginning of the spermatogenetic period. Moreover, the spermatogenetic activity during summer is considerably higher than that obtained by the injection of a summer pituitary extract.

In our opinion the results of these experiments justify the conclusion that the seasonal changes in spermatogenetic activity in *Rana temporaria* are at least partly the result of changes in the sensitivity of the germinal epithelium to gonadotrophic hormone. Some other of our experiments (P. G. W. J. van Oordt, 1956 a, b), to be discussed later, and dealing with the effect of gonadotrophin injections and changes in the ambient temperature, confirm this conclusion.

The influence of the preoptic nucleus

The question whether the preoptic nucleus of the hypothalamus affects the gonadotrophin production of the pituitary, and, therefore, spermatogenesis was studied in 1924 and 1935 by Houssay and his co-workers in *Bufo arenarum*. It was found that although lesions of the infundibulo-tuberal region are followed by degeneration of central and ventral parts of the pars distalis (Houssay, Biasotti & Sammartino, 1935), they do not lead to atrophy of the testes (Giusti & Houssay, 1924). The absence of any effect on spermatogenesis could be ascribed to regeneration of the pars distalis, since after 25-35 days the circulation of the gland had become normal (Houssay & Giusti, 1930 ; Lascano-González, 1935).

Similar results were obtained by Mazzi (1950 a, b ; 1952 a ; 1958 a, b) with *Triturus cristatus*. Lesions of the median eminence and the hypophyseal portal vessels did not modify the pars distalis and spermatogenesis if regeneration of the portal vessels occurred. If, however, the median eminence and the hypophyseal portal vessels were interrupted by a piece of thin parchment paper or a thin piece of celloidin, the gonadotrophin-producing cells in the pars distalis decreased in number and spermatogenesis was impaired. Section

of the preoptic-hypophyseal tract did not cause degeneration of cells in the preoptic nucleus, and if the medium eminence was situated rostral to the piece of celloidine a new neural lobe was formed. Comparable results have been obtained by Jørgensen, Rosenkilde & Wingstrand (1956) in *Bufo bufo*.

Since cauterisation of the preoptic area of the hypothalamus (Mazzi, 1952 b) has very much the same effects upon the pituitary as interruption of the portal vessels, it can be deduced from Mazzi's experiments that the preoptic nucleus in *Triturus cristatus* produces an agent necessary for the gonadotrophic function of the pars distalis, this agent being transported to that part of the pituitary via the preoptic-hypophyseal fibres ending in the median eminence and then via the hypophyseal portal vessels. It is not unlikely that seasonal changes in the activity of the neurosecretory system, as found by Mazzi (1947) in *Triturus cristatus* and by Wilson, Weinberg & Bern (1957) in *Hyla regilla*, have something to do with changes in the production and secretion of the neurohumoral substance which enhances the function of gonadotrophic cells in the pars distalis.

The importance of the hypophyseal portal vessels for the gonadotrophic function of the pars distalis is also shown by experiments carried out with other amphibian species. As early as 1935 Vunder (cited by Vivien & Schott, 1957) found that the pituitary of the axolotl, autografted in an heterotopic site, can overcome the effects of hypophysectomy only for some months. Pasteels Jr. (1957) observed that autotransplantation of the adenohypophysis of juvenile *Pleurodeles waltlii* on to the palatal epithelium leads to an involution of the thyrotrophs. The gonadotrophs change into mucin-producing cells and no development of the gonads take place.

Vivien & Schott (1957) autografted the pars distalis of a number of adult common frogs (*Rana temporaria*) on to the levator bulbi muscle. After six months, during the summer season, the well-vascularized glands histologically resembled normal, quiescent winter pituitaries, and the gonads were identical with those of hypophysectomized controls. On the other hand, the pituitary and gonads of the frogs were completely normal for the summer season if the pars distalis had been autografted on to the median eminence and was vascularized by hypophyseal portal vessels. These experiments complemented those of Jacobsohn & Jørgensen (1956), who found that the hypophyseal portal system is necessary to prevent hyperkeratinisation of the skin in *Bufo bufo*.

The influence of male sex hormones

In comparison with the many studies on the influence of the pituitary gland on spermatogenesis, little attention has been paid to the possible role of testosterone in the internal regulation of the spermatogenetic cycle of Amphibia. This may be partly due to the fact that oils, and therefore also the often used oil solutions of steroid hormones, block the lymph hearts of Amphibia, causing oedema and the death of the animals (*cf.* Valle, Penhos & Houssay, 1952). Moreover, in some, particularly the earlier, experiments concerning the effect of testosterone no influence was found on spermatogenesis, although secondary sex characters were affected (*cf.* Puckett, 1939 ; Greenberg, 1942 ; Galgano, 1942 d).

Blair (1946), on the other hand, studying the results of testosterone propionate and androsterone injections in juvenile *Bufo fowleri*, found a slight stimulation of spermatogenetic activity. Penhos (1953, 1956) reports that testosterone propionate injections are followed by an increase in testis weight in juvenile *Bufo arenarum*. Also Iwasawa (1957), using *Triturus pyrrhogaster*, found that testosterone stimulates spermatogenesis. These authors, however, do not state whether the whole spermatogenetic process or only part of it is stimulated by the male sex hormone.

This problem was studied by Cei, Andreozzi & Acosta (1955) in *Leptodactylus chaquensis*. High doses of testosterone propionate appeared to cause degeneration of secondary spermatogonia and primary spermatocytes, and to impede the mitotic capacity of the primary spermatogonia. In some experimental animals, however, the development of secondary spermatocytes and spermatids was stimulated by the hormone.

Somewhat the same results were obtained in our laboratory (P. G. W. J. van Oordt & Basu, 1960) by treating *Rana temporaria* with testosterone pellets, implanted in the dorsal lymph sac. It was found that the male sex hormone impairs the formation of secondary spermatogonia from primary spermatogonia, but does not affect the mitotic capacity of the latter cell type. Moreover, degeneration of primary spermatocytes was not found, and it is believed that the development of this cell type and of secondary spermatocytes and spermatids is stimulated by testosterone. The secondary spermatogonia degenerated during the testosterone treatment.

Since hypophysectomy in *Rana temporaria* also mainly affects the production and development of secondary spermatogonia (P. G. W. J. van Oordt, 1956 a), and in *Leptodactylus chaquensis* degeneration of these cells following testosterone propionate injections can be prevented by simultaneous injections of serum gonadotrophin (Cei *et al.*, 1955), it seems likely that in these species testosterone affects the gonadotrophin production of the pituitary. We have recently been able to confirm this hypothesis, as far as the damaging effect of testosterone on secondary spermatogonia is concerned (P. G. W. J. van Oordt & Faddegon, unpublished results). It appears that the spermatogenetic activity in hypophysectomized common frogs, induced by injection of serum gonadotrophin, is not markedly altered when testosterone propionate is administered together with the gonadotrophic hormone preparation. We also observed changes in the gonadotrophin producing cells of *Rana temporaria*, following testosterone treatment (P. G. W. J. van Oordt, unpublished results). These results show that testosterone does not modify the sensitivity of the spermatogonia to gonadotrophic hormone, but affects the secretion of this hormone by the pars distalis. It is not unlikely that the stimulation of the spermatocytes and spermatids caused by testosterone follows different pathways.

The findings concerning the effect of testosterone on the gonadotrophin secretion in *Rana temporaria* are in accordance with the fact that castration causes hyperactivity of pituitary gonadotrophs. This was found by Cordier (1953) in *Xenopus laevis*, by Zuber-Vogeli (1953) in *Bufo bufo* and by P. G. W. J. van Oordt (unpublished results) in *Rana temporaria*. It must, however, be said that the effect of castration on the amphibian pituitary only appears long

after the operation, and that in short term experiments no changes in the pituitary cytology and gonadotrophin content were found (*cf*, Novelli, 1932 ; Adams & Mayo, 1936 ; Zahl, 1937).

This brings us to the question whether male sex hormone can be supposed to exert any influence on spermatogenesis in Amphibia under natural conditions. It is generally believed that male sex hormone is produced by interstitial Leydig cells (Anura) or glandular tissue filling up the empty ampoules (Urodela) of the testis. This opinion is largely based on the fact that castration leads to atrophy of the secondary sex characters and that cyclical changes in the development of the secondary sex characters correspond to seasonal changes in the secretory activity of the interstitial cells. Moreover, histochemical investigations by Ashbel, Cohen & Seligman (1951) and by Burgos (1955) using *Rana pipiens*, by Niwelińsky (1954) using *Xenopus laevis*, and by de Kort, Berendsen, Westra & P. G. W. J. van Oordt (unpublished results) using *Rana esculenta*, indicate that ketosteroids are formed in the interstitium of the testis.

In practically all Amphibia, however, the period of most intense spermatogenetic activity never corresponds with secretory activity of the interstitial cells. It is, therefore, not likely that the male sex hormone normally stimulates the development of spermatocytes and spermatids. On the other hand, it is not impossible that in Amphibia with a discontinuous spermatogenetic cycle the male sex hormone is one of the factors preventing the onset of spermatogenesis during the resting period and the spermiation period.

The influence of some other internal factors

Apart from the hypothalamus, the pars distalis of the pituitary and the interstitial Leydig cells in the testis, few other organs have been tested for their influence on the spermatogenetic cycle in Amphibia.

Taylor (1936) suggests that in *Triturus torosus* the sensitivity of many tissues, including the testes, decreases after thyroidectomy. The assumption that thyroid hormones influence the testis is not supported, however, by the results of Joel, d'Angelo & Charipper (1949). The authors did not find any influence of thiouracil and thyrotrophin injections on the testes of *Rana pipiens* during winter. It may be added that thyroid hormones influence neither spermiation in *Bufo arenarum* (Burgos & Mancini, 1947), nor mating behaviour in *Rana temporaria* (Smith, 1951).

Rhythmical changes in body weight, weight of the corpora adiposa, blood sugar level, glycogen content of the liver, etc. have been found in *Bufo arenarum* (*cf.* Houssay, 1949) and in *Rana temporaria* (du Bois, 1927 ; Smith, 1950, 1952, 1954). Such findings indicate that other internal factors than hormones might also be of importance for the regulation of the sexual cycle in Amphibia.

It is, for example, not unlikely that the increase in sensitivity of the germinal epithelium of *Rana temporaria* to gonadotrophic hormone between January and April (*cf.* G. J. van Oordt, Sluiter & P. G. W. J. van Oordt, 1951 ; P. G. W. J. van Oordt, Sluiter & G. J. van Oordt, 1952) can be ascribed to a sharp rise in general metabolism taking place within this period (*cf.* Krogh, 1904 ; Dolk & Postma, 1927). This rise in general metabolism corresponds with the end of

hibernation, which according to Barthelemy (1926) in *Rana temporaria* and to Holtzapfel (1937) in *Rana pipiens* and *Rana catesbeiana* does not depend on the environment, but on intrinsic factors determining the seasonal cycle of these Amphibia.

REGULATION OF THE SPERMATOGENETC CYCLE BY EXTERNAL FACTORS

The influence of feeding

In the introduction to this paper it has been stated that investigations into the influence of climatic factors on spermatogenesis and breeding of Amphibia date from the year 1924, when Witschi studied the importance of such factors for the regulation of the spermatogenetic cycle in *Rana temporaria*. This is certainly true as far as a detailed study of effects of external factors on the different processes of the spermatogenetic cycle is concerned. However, basic interest in the regulation of the sexual cycle by external factors is actually of older date. For example, in the nineteenth century, investigators tried to determine the optimal conditions for breeding Amphibia in the laboratory. To that end the effects of feeding, light, rainfall and temperature were studied.

Generally speaking it can be said that in most Amphibia the sexual cycle becomes irregular if the animals are kept under laboratory conditions, and not uncommonly gametogenesis and breeding stop in Amphibia kept in the laboratory for a long period. Such results of captivity have often been ascribed to abnormal feeding. Starvation was found to impair testicular functions in *Rana temporaria* (Nussbaum, 1906 ; Cei, 1944 ; Sluiter, G. J. van Oordt & Grasveld, 1950), in *Triturus alpestris* (Champy, 1921), in *Xenopus laevis* (Alexander & Bellerby, 1935, 1938), in *Discoglossus pictus* (Kehl, 1944), in *Bufo arenarum* (Penhos & Cardeza, 1950) and in *Bufo bufo* (Zander, 1951). According to Penhos & Cardeza (1950), however, the same damaging effect upon the testes of *Bufo arenarum* is seen after forced feeding.

In our opinion these observations show that unfavourable conditions, in this case starvation or too much food, ultimately leading to the death of the animal, impair gametogenesis and breeding in Amphibia. The fact that feeding is indeed not a primary factor in the regulation of the spermatogenetic cycle follows from the results obtained by Ifft (1942) in *Triturus viridescens* and by Cei (1942) in *Triturus alpestris*. In both species spermatogenesis was not impaired in animals that were starved for a considerable length of time. We have also obtained spermatogenesis and spermiation in common frogs (*Rana temporaria*) which were not fed during the experiments. It was, moreover, impossible to induce spermatogenetic activity by an ample supply of food in common frogs during the resting period (P. G. W. J. van Oordt, 1956 a).

The influence of light

It has been supposed that light stimulates spermatogenesis in Amphibia (*cf.* March, 1937 ; Spaul & Gladwell, cited by Bullough, 1939), but the results of many investigations do not support this view. It has been possible, for example, to induce spermatogenesis by changing climatic conditions, but without increasing light period or light intensity. This has been done by Galgano (1931-1936) in *Rana esculenta*, by Galgano & Falchetti (1940), by

Galgano (1941-1947) and by Mazzi & Galgano (1949) in *Triturus cristatus*, by Ifft (1942) in *Triturus viridescens*, by Cei (1942 a) in *Geotriton* (=*Spelerpes*) *fuscus*, by Cei (1942 b) in *Triturus alpestris*, by Cei (1944) in *Rana graeca*, and by Galgano & Lanza (1951) and by P. G. W. J. van Oordt (1956 a, b) in *Rana temporaria*.

Moreover, Ifft (1942) found that neither a steadily increasing light period nor constant light had any effect on the spermatogenetic activity of *Triturus viridescens*. Similar results were also obtained in our laboratory (P. G. W. J. van Oordt, 1956 a). Spermatogenesis could not be induced in *Rana temporaria* during the resting period by exposing the animals to long daily periods of electric illumination.

Spermatogenesis is also completely normal when *Triturus viridescens* (Ifft, 1942) or *Rana temporaria* (Cei, 1944: P. G. W. J. van Oordt, 1956 a) are kept in complete darkness for long periods. Also in *Leptodactylus ocellatus* the absence of light does not affect spermatogenetic activity (Cei, 1948). According to Tobler (1947) spermatogenesis is not even retarded in *Triturus alpestris*, raised from the beginning in the dark. These arguments together with the fact that various amphibian species live in dark places during the spermatogenetic period make it appear quite unlikely that light has any direct effect on regulation of spermatogenesis in Amphibia.

This does not mean that light has no influence at all on the spermatogenetic cycle. Many authors have stated that light induces spermiation and breeding behaviour in Amphibia. It is beyond the scope of this paper, however, to discuss this point.

The influence of rainfall

There appears to be a lack of experimental evidence indicating an effect of rainfall on the spermatogenetic process in Amphibia. However, many investigators (*e.g.* Miller & Robbins, 1955) have carried out studies on the relation of rainfall to breeding behaviour and migration. These studies also appear to be beyond the scope of the present discussion.

The influence of temperature

The importance of temperature for the regulation of the spermatogenetic cycle has been stated by many investigators in many species. Confining ourselves to the results of experiments on the effect of temperature on spermatogenesis, it has to be said that this effect seems to be different in different species.

Galgano (1931-1936) found that in *Rana esculenta* the spermatogenetic activity can always be induced by raising the environmental temperatures to the normal summer temperatures of the frogs' habitat. Reciprocally, spermatogenesis always stops when the temperature decreases to the normal autumn and winter level. It is, therefore, clear that the resting period of the spermatogenetic cycle in *Rana esculenta* is caused solely by the unfavourable autumn and winter temperatures, and for that reason the spermatogenetic cycle in the edible frog is discontinuous and not continuous. Galgano, there-

fore, rightly pointed out that this type of spermatogenetic cycle should be called " potentially continuous ".

Such potentially continuous spermatogenetic cycles have also been found by Galgano & Falchetti (1940), by Galgano (1941-1947), and by Mazzi & Galgano (1949) in *Triturus cristatus carnifex*, by Ifft (1943) in *Triturus viridescens* and by Cei (1942 a, b ; 1944) in *Geotriton* (=*Spelerpes*) *fuscus*, in *Triturus alpestris* and in *Rana graeca*. Also in these species an increase of the external temperature to about 20°C during the resting period is followed by a strong spermatogenetic activity, whereas low temperatures during summer cause degeneration of the spermatogenetic elements and impairment of the spermatogenetic activity.

In addition to these European Amphibia, the South-American frog *Leptodactylus ocellatus typica* also appears to have a potentially continuous spermatogenetic cycle. According to the studies of Cei (1948, 1949 a) and Rengel (1950) the process of spermatogenesis in this form has a lower limit of temperature tolerance, which normally causes interruption of spermatogenesis during winter, and a high limit of temperature tolerance ($\pm$ 30°C), which may bring about a second cessation during summer months. Animals which are kept within these limits show a continuous spermatogenetic cycle.

In *Rana temporaria* the situation is completely different, as was shown as early as 1924 by Witschi in his investigations on the spermatogenetic cycle of two geographical races of the common frog. One, the so-called " early race ", is found in lower parts of Western Europe, while the other, the so-called " late race " is found in the Alps and in Northern Europe. In the former, spermatogenesis takes place from May till September and is followed in March by the breeding period. In the latter, breeding does not start before May, but spermatogenesis begins in April and ends in August. It is clear that in both races the spermatogenetic cycle is very well adapted to the climatic conditions of the habitat. The remarkable thing is, however, that when Witschi brought animals from the Alps to lower regions the spermatogenetic cycle did not change at all. Witschi, therefore, concluded that in *Rana temporaria* the spermatogenetic cycle is to a large extent independent of environmental factors and consequently depends on an internal rhythm determined by genetic factors.

Cei (1942 c - 1944) arrived at the same conclusion when he found that in common frogs belonging to the early race no spermatogenetic activity could be induced by exposing them to high temperatures during the resting period of the spermatogenetic cycle. Less clear results were obtained with specimens belonging to the late race kept at high temperatures during this period. In most of these animals no precocious onset of spermatogenesis was observed, whereas in others cell nests in all stages of development were found. As the number of those cell nests, however, remained below the number normally found during the spermatogenetic period, Cei is of opinion that any influence of temperature on the normal spermatogenetic cycle during the resting period is satisfactorily disproved by his results.

These experiments were repeated in our laboratory (P. G. W. J. van Oordt, 1956 a, b) with common frogs belonging to the early race. Cei's results, *i.e.* no spermatogenetic activity in frogs kept at high temperatures, were confirmed

by experiments carried out during the first part of the resting period. On the other hand, high temperatures appeared to induce spermiation in the experimental animals. In other words, the resting period of the spermatogenetic cycle was shortened by the high temperatures and the testes entered the spermiation period.

Other experiments, however, carried out by Galgano & Lanza (1951) and by ourselves (P. G. W. J. van Oordt, 1956 a, b) during the second half of the resting period with frogs belonging to the early race, clearly show that high temperatures not only induce spermiation, but also cause a very strong spermatogenetic activity.

These results are comparable with those obtained by Cei (1944) with common frogs of the late race brought from their original habitat to warmer regions at the beginning of the spermatogenetic period. In these animals, as with the above, the higher temperatures enhanced the spermatogenetic activity. It was observed, however, that this spermatogenetic activity did not continue during the whole summer. In early July the germinal epithelium entered the resting period and remained in it, even though the environmental temperatures were high.

Cei (1944) also investigated the influence of abnormally low temperatures on spermatogenesis by keeping frogs in a refrigerator for ten days. This treatment had no effect on the spermatogenetic activity. In our laboratory (P. G. W. J. van Oordt, 1956 a), however, it was found that after exposure to a temperature of 5°C for nearly two months from the beginning of the spermatogenetic period, spermatogenesis in common frogs of the early race was almost completely absent.

In our opinion these results, obtained by Cei, by Galgano & Lanza and by ourselves, justify the conclusion that in *Rana temporaria* the resting period is not initiated by low temperatures, but by a genetically determined, internal mechanism. The onset of spermatogenesis, however, does not depend on this internal rhythm, but on environmental temperatures. It can, therefore, be concluded that, contrary to the situation in many Amphibia, the spermatogenetic cycle of *Rana temporaria* is really discontinuous, and it is proposed to call this cycle a " discontinuous cycle *sensu stricto* ".

Such a discontinuous spermatogenetic cycle *s.s.* was also found by Cei (1944) in *Rana arvalis* and *Rana dalmatina*. In *Rana arvalis* high temperatures caused hardly any spermatogenetic activity during the resting period. In *Rana dalmatina* spermatogenesis was only found in some of the animals exposed to high temperatures during the end of the resting period and the spermiation period.

Concerning the influence of temperature on the regulation of spermatogenesis in Amphibia with a continuous spermatogenetic cycle very little is known. As mentioned above (page 30), in *Telmatobius schreiteri* (Cei, 1949 b) and *Hyla raddiana andina* (Caruso, 1949) spermatogenesis does not stop during the cold winter period. Rengel (1950) found that in *Leptodactylus ocellatus reticulata* spermatogenesis is not impaired when the animals are exposed to temperatures higher than 30°C, a range which was found to be too high for spermatogenesis in *Leptodactylus ocellatus typica*. It is, therefore, not impossible that in some

of the species with a continuous spermatogenetic cycle this cycle is more or less independent of the environmental temperature.

THE COOPERATION OF THE INTERNAL AND THE EXTERNAL FACTORS IN THE REGULATION OF THE SPERMATOGENETIC CYCLE

The internal and external factors which are indispensable for the spermatogenetic activity, or at least are of some importance to Amphibia in the regulation of the spermatogenetic cycle, can be divided into three groups (*cf.* Fig. 1).

One group of what may be called " final factors " includes the gonadotrophic activity of the pituitary gland, and the sensitivity of the germinal epithelium to gonadotrophic hormone. These factors act directly on the spermatogenetic cells to bring about the onset and maintenance of the spermatogenetic processes.

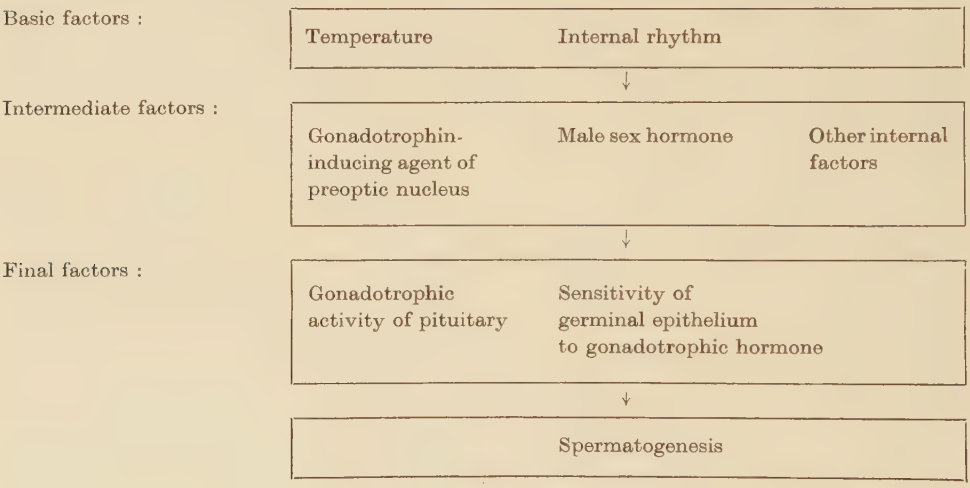

Fig. 1—.Groups of internal and external factors necessary for the regulation of amphibian spermatogenesis. The internal rhythm does not determine spermatogenesis in Amphibia with a continuous or a potentially continuous spermatogenetic cycle.

Each of these final factors is influenced by what may be called " intermediate factors ", *i.e.* the gonadotrophin-inducing agent of the preoptic nucleus, the male sex hormone and other internal factors. It has been pointed out above that the gonadotrophin-inducing agent of the preoptic nucleus is necessary for the gonadotrophic function of the pituitary gland. The latter can also be influenced by the male sex hormone. Other internal factors such as the general metabolism may influence the sensitivity of the germinal epithelium to gonadotrophic hormone.

In all Amphibia spermatogenetic processes are regulated by one so-called " basic factor ", the environmental temperature ; in some also by another basic factor, the internal rhythm. Little is known about the effect of the temperature on the intermediate factors. Since the preoptic nucleus is indispensable for the gonadotrophic activity of the pituitary, Mazzi & Galgano [1949 ; *cf.* also Galgano & Mazzi (1951) and later publications by Mazzi] suppose that an effect of temperature on the gonadotrophic activity of the pituitary

must be transmitted via this part of the hypothalamus. It has also been found that in *Rana esculenta* (Galgano, 1931 - 1936), *Triturus cristatus* (Galgano, 1941 - 1947) and *Rana temporaria* (Cei, 1944) the male secondary sex characters atrophy when the animals are kept at high temperatures during winter. Moreover, de Kort, Berendsen, Westra & P. G. W. J. van Oordt (unpublished results) observed involution of the interstitium in *Rana esculenta* kept at high temperatures, and also disappearance of keto-groups, which are present in the cytoplasm of interstitial cells of normal animals during winter, and which indicate the presence of keto-steroids.

The influence of temperature on the final factors has been studied in two species with a potentially continuous spermatogenetic cycle, namely *Triturus cristatus* and *Rana esculenta*. Galgano (1941) injected *Triturus cristatus* with mammalian pituitary extract in winter, spring and summer. During the resting period of the spermatogenetic cycle this treatment only caused spermatogenesis when the newts were kept at high temperatures. Similar experiments carried out by Galgano (1942 d) with *Rana esculenta* had very much the same results. According to Galgano these results show that low winter temperatures make the germinal epithelium insensitive to gonadotrophic hormone.

The fact that in *Triturus cristatus* the gonadotrophin secretion of the pituitary is also impaired by low winter temperatures follows from the observations of Mazzi (1949, 1950 a, b) and of Mazzi & Pieri (1949). These authors found that in newts kept at high temperatures during the resting period, the cytohistology of the pars distalis changes from the inactive winter stage to the stage of high activity, normally found during the spermatogenetic period.

Considering the results of Galgano and Mazzi, it can be concluded that in Amphibia with a potentially continuous spermatogenetic cycle, this cycle is completely controlled by environmental temperatures, which affect both the gonadotrophin secretion of the pituitary and the sensitivity of the germinal epithelium to gonadotrophic hormone. The seasonal changes in the temperature divide the spermatogenetic cycle into two periods (*cf.* Fig. 2). During the warmer season the high temperatures (temperature +) induce the secretion of gonadotrophic hormone and sensitize the germinal epithelium to this hormone. This results in the formation of secondary spermatogonia, which develop into spermatocytes, spermatids and sperm cells (*cf.* Galgano, 1935, 1945). At the end of the warmer season the decreasing temperature (temperature —) impairs both of the final factors and the formation of new secondary spermatogonia stops. The secondary spermatogonia, spermatocytes and spermatids either remain quiescent or degenerate during the colder season.

The effect of temperature and internal rhythm on the final factors of Amphibia with a discontinuous spermatogenetic cycle *s.s.* has only been studied in one species, *viz.* in *Rana temporaria* (P. G. W. J. van Oordt, 1956 a, b). Experiments were carried out at three different stages of the spermatogenetic cycle : the first part of the resting period, the latter part of the resting period and the first part of the spermatogenetic period.

During the first part of the resting period serum gonadotrophin was injected into intact and hypophysectomized frogs, kept at high or normal winter temperatures. The gonadotrophin appeared to induce spermatogenesis only

in intact frogs kept at high temperatures, but not in any of the hypophysectomized frogs nor in the intact frogs kept at low temperatures. The absence of spermatogenetic activity in the hypophysectomized frogs injected with the

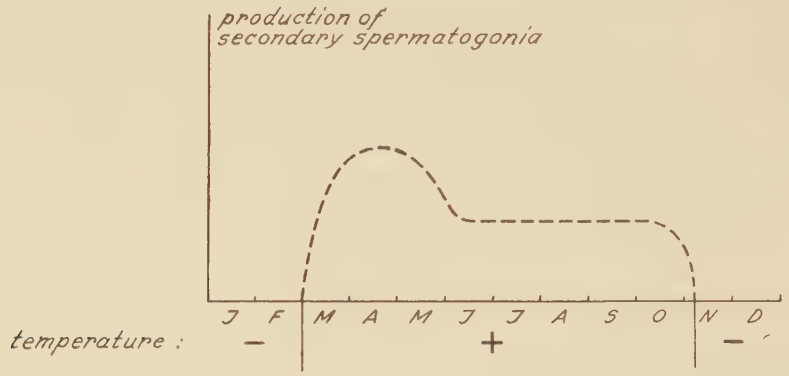

Fig. 2.—The production of secondary spermatogonia in Amphibia with a potentially continuous spermatogenetic cycle, and its regulation by environmental temperatures. For explanation see text.

gonadotrophin shows that during the first part of the resting period the sensitivity of the germinal epithelium to gonadotrophic hormone is very low, and that the internal rhythm prevents the increase of this sensitivity by high temperatures. The fact that of all intact frogs injected with serum gonadotrophin, only in those kept at high temperatures was spermatogenesis found, shows that in the latter animals the amount of circulating gonadotrophic hormone is larger than in animals kept at low temperatures. In other words, the gonadotrophin secretion of the pituitary was increased by the high temperatures.

During the latter part of the resting period serum gonadotrophin or frogs' pituitary extract was injected into hypophysectomized animals. Only in those animals which were kept at high temperatures did gonadotrophin preparations cause the production of many cell nests containing secondary spermatogonia. This confirms the observation by G. J. van Oordt, Sluiter & P. G. W. J. van Oordt (1951 ; *cf.* also P. G. W. J. van Oordt, Sluiter & G. J. van Oordt 1952) that during the latter part of the resting period the sensitivity of the germinal epithelium to gonadotrophic hormone is relatively low. It shows, moreover, that this low sensitivity is no longer fixed by the internal rhythm, but can be increased by high temperatures.

Frogs' pituitary extract injected into intact frogs, kept at high temperatures during the latter part of the resting period, increased the development of the spermatogenetic elements already formed under the influence of high temperatures, but did not induce the multiplication of the spermatogonia (*cf.* also Galgano & Lanza, 1951). The same treatment, however, did result in the production of some cell nests containing secondary spermatogonia in intact frogs kept at low temperatures. This means that after the production of secondary spermatogonia, the remaining primary spermatogonia are refractory to the influence of the gonadotrophic hormone. This refractoriness of the pri-

mary spermatogonia is, therefore, the factor through which the internal rhythm manifests itself at the end of the spermatogenetic period and during the first part of the resting period.

Homologous gonadotrophin, injected into hypophysectomized frogs kept at low or at normal summer temperatures during the first part of the spermatogenetic period, caused the formation of cell nests containing secondary spermatogonia. Since no significant difference was found between the frogs kept at low temperatures and those kept at normal summer temperatures, it can be concluded that the low temperatures did not decrease the sensitivity of the germinal epithelium to gonadotrophic hormone. It can also be deduced from these results that during the first part of the spermatogenetic period the internal rhythm manifests itself through the relatively high sensitivity of the primary spermatogonia to gonadotrophic hormone. The absence of spermatogenesis in uninjected intact frogs kept at low temperatures during this period must, therefore, be ascribed to a decrease in the gonadotrophin secretion of the pituitary.

Summarizing these results, it can be said that in *Rana temporaria* the gonadotrophin secretion of the pituitary depends upon environmental temperatures. The temperature and internal rhythm together determine the sensitivity of the germinal epithelium to gonadotrophic hormone. The seasonal changes in the two basic factors divide the spermatogenetic cycle into four periods (*cf.* Fig. 3). From January till April the internal rhythm allows the temperature to affect the sensitivity of the primary spermatogonia to gonadotrophic hormone (internal rhythm +). The temperature, however, is too low to induce gonado-

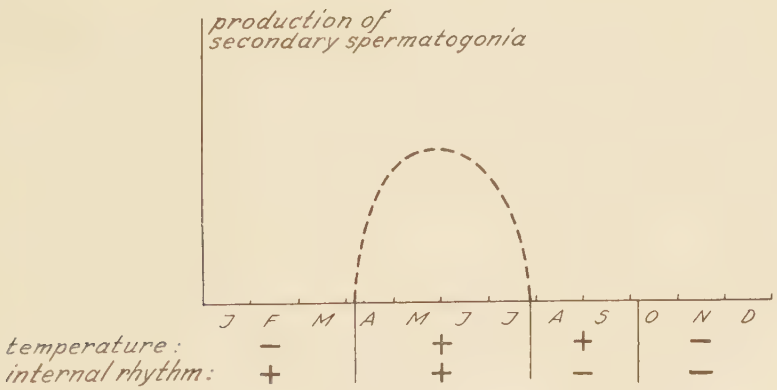

Fig. 3.—The production of secondary spermatogonia in Amphibia with a discontinuous spermatogenetic cycle *s.s.*, and its regulation by environmental temperatures and the internal rhythm. For explanation see text.

trophin secretion of the pituitary or to increase the sensitivity of the germinal epithelium to gonadotrophic hormone (temperature −). The production of secondary spermatogonia is, therefore, impossible during this period. At the beginning of the spermatogenetic period in April the increasing temperature stimulates both final factors. From April till the end of July high temperatures

induce gonadotrophin secretion of the pituitary (temperature +) and the internal rhythm keeps the primary spermatogonia sensitive to this hormone (internal rhythm +). During this period the primary spermatogonia, therefore, pass over into secondary spermatogonia (*cf.* van Oordt, 1956a). From the end of July all primary spermatogonia are refractory to gonadotrophic hormone (internal rhythm −) and the formation of secondary spermatogonia stops. The temperatures, however, are high enough to cause gonadotrophin production (temperature +). In October the temperatures also become too low (temperature −) to induce the secretion of gonadotrophic hormone. From October till January low temperatures together with the insensitivity of the primary spermatogonia to gonadotrophic hormone (internal rhythm −) prevent the onset of spermatogenetic activity.

Comparing the potentially continuous and the discontinuous spermatogenetic cycles, it appears that both are regulated by the same factors. Nevertheless, in Amphibia with a potentially continuous spermatogenetic cycle secondary spermatogonia are formed during the entire warmer season, whereas in Amphibia with a discontinuous spermatogenetic cycle *s.s.* the production of secondary spermatogonia is confined to a shorter period. This important difference must be ascribed to the fact that in Amphibia with a discontinuous spermatogenetic cycle *s.s.* all primary spermatogonia are highly insensitive to gonadotrophic hormone during large parts of the year, whereas in Amphibia with a potentially continuous spermatogenetic cycle there are always primary spermatogonia which can respond to gonadotrophic hormone and pass over into secondary spermatogonia. The latter is in fact also the case in Amphibia with a continuous spermatogenetic cycle.

SUMMARY

A review has been given of the literature dealing with the regulation of the spermatogenetic process in Amphibia by internal and external factors.

The factors which influence spermatogenesis have been divided into three groups, *i.e.* the basic factors, the intermediate factors and the final factors (*cf.* Fig. 1).

The intermediate factors and the final factors are the same in all species investigated. Temperature is the basic factor which determines spermatogenesis in all Amphibia.

It is the presence of the internal rhythm as one of the basic factors regulating spermatogenesis in Amphibia with a discontinuous spermatogenetic cycle *sensu stricto* which makes this spermatogenetic cycle fundamentally different from the potentially continuous spermatogenetic cycle (*cf.* Figs. 2 and 3) or the continuous spermatogenetic cycle.

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THE ENVIRONMENT, CYCLICAL REPRODUCTIVE ACTIVITY AND BEHAVIOUR IN BIRDS

BY

A. J. MARSHALL

Department of Biology, Monash University, Melbourne, Australia.

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INTRODUCTION

If we look at the world, rather than merely the European and North American bird fauna, we will see very clearly the operation, and the utility, of an internal rhythm of reproduction. It is, in fact, the most important single, and only universal, factor in the regulation of breeding seasons, including migration (Marshall, 1958). By internal rhythm I mean the metrical succession of correlated events that are variously under endogenous and exogenous control. At the outset, too, I must emphasize that although we have in it something akin to a clock, it must be nevertheless likened to an imprecise, chain-store variety that has to be " corrected " by external factors at least once during each annual cycle.

The reason why the existence of this partly endogenous rhythm is not more widely accepted is that almost all investigators have restricted their attention to temperate zone birds, and have allowed the spectacular influence of exogenous photostimulation in many such species to obscure any clear view of the basic mechanism underlying it.

Rowan's pioneer work led to an era of photostimulation experiments which have been productive of a great deal of vital information. Most people do not realize that it was this great innovator, in fact, who was the first (as far as I can gather) to postulate the existence of an internal rhythm of reproduction. Rowan (1926, 1927), at the height of his photostimulation work in Canada, concluded that some kind of rhythm must be operative in the regulation of the breeding cycles of equatorial species. In Africa, Moreau (1931), with whom Rowan corresponded, came to the same conclusion. Later, F. H. A. Marshall (1936, 1937), Baker & Baker (1936) and Baker (1938) all produced *prima facie* evidence of the operation of an endogenous rhythm. Baker & Baker (1936) were insistent that such a rhythm could not be indefinitely self-regulatory and that external stimuli must operate from time to time to synchronize it with

the cycle of the sun, i.e. with the seasons. Marshall (1949a, 1951a, 1955) produced additional information and suggested that, notwithstanding the vital influence of photostimulation in many species, the most positive timing and regulating (as distinct from initiating and accelerating) factors are the external stimuli in the traditional breeding grounds that operate, via the female exteroceptors, only when the environment becomes seasonally suitable for reproduction.

Photoperiodicity does not, even in most temperate zone birds, control the *time of breeding*. It does however, initiate the " spring " (often late-winter) sexual processes in very many temperate zone species at a time sufficiently early to allow an adequate period for the acquisition of territory, display and the other hormone-induced processes necessary, along with additional external stimuli, for successful reproduction. In probably hundreds of species living in tropical, and periodically dry (not necessarily desert) regions, light has no influence whatever. We shall see that *light is important only in species for which it is important that light shall be important.*

THE AVIAN SEXUAL CYCLE

I have recently (Marshall, 1959, 1960) discussed this at some length and I will try not to be unduly repetitive in regard to data or references.

The partly endogenous and partly exogenous rhythm of reproduction, involving successive phases of post-nuptial *regeneration* (reflected in a sudden loss and subsequent recovery of breeding function), *acceleration* (characterized by sex hormone production and gametogenesis) and *culmination* (involving ovulation and insemination) can be considered as a sort of cog-wheel which is engaged at various times by changing environmental "teeth" that are helpful, or otherwise, to reproductive success and to the existence of the species.

The internal rhythm is the primary seasonal *initiator*. As young birds mature, so does their neuro-endocrine apparatus. Young budgerygahs (*Melopsittacus undulatus*) (Vaugien, 1953) and zebra finches (*Peophila castanotus*) (Marshall & Serventy, 1958) will produce bunches of spermatozoa within sixty days in almost total darkness if given adequate warmth, food and water. These are temperate-zone birds which live in dry, but not desert, areas. In the zebra finch, and numerous other species, reproduction can occur within six months of hatching. Likewise, among adults the internal rhythm is the seasonal initiator. Until the post-nuptial regeneration phase is past, the male certainly, and the female probably, is uninfluenced by the external stimuli that would cause gametogenesis at other periods of the cycle. After the spontaneous progression from regeneration to acceleration (when sex hormones are once more liberated and gametogenesis is again possible) the neuro-endocrine machinery of each individual comes under the constant influence of two sets of external factors as follows :

accelerators $\leftrightharpoons$ *inhibitors*

These are antagonistic. The cycle hastens, slows or sometimes stops altogether, depending upon the factors currently presented to it by the changing environment. The more unstable the climate, the more obvious the truth

of this becomes. For example, an accelerator of the sexual cycle of many temperate-zone birds is light. An inhibitor of the cycle of most species is cold. In an unusually cold spring the effects of light are nullified and so reproduction is delayed. Among water-birds and many others a frequent inhibitor is the lack of a safe and traditional nesting site. If the seasonal appearance of such a site is delayed sufficiently long in an habitually single-brooded species, internal changes occur that lead to metamorphosis of the testis and so reproduction is frustrated for that particular season. Other accelerators are the innate behavioural reactions induced in each sex by their gradually changed endocrinology, as well as by the presence of the displaying mate. Yet ovulation, even after the first egg is laid, may be inhibited by sudden cold, fear or other traditional inhibitor.

Regeneration phase. This comes immediately after reproduction in single-brooded species and after the final ovulation of the season in plural-breeded ones. It varies in duration between species. It is a period in which the neuro-endocrine apparatus does not respond to photostimulation, hence the use of the term " refractory period " by Bissonette & Wadlund (1932). It is accompanied by the almost complete metamorphosis of the testes. After the seasonal exhaustion, or near exhaustion, of the interstitium, the Sertoli and germ-cell cytoplasm are converted into a mass of cholesterol-positive lipid material which appears to give rise to progestins (Marshall 1949b, Lofts & Marshall 1959). The same effects can be induced by the injection of prolactin (Lofts & Marshall 1956), or by the removal of the anterior lobe of the pituitary gland (Coombs & Marshall 1956). Automatically, and even after the removal of the hypophysis, the interstitium rhythmically regenerates and the tubule lipids disappear. The rate of disappearance varies between species. Yet there is no suggestion that the interstitium could *secrete* in the absence of the hypophysis.

Although the testis is thrown out of spermatogenetic productivity during the regeneration phase, and there is evidence (Riley & Witschi 1938) that also the ovary becomes " refractory ", these events are governed from elsewhere (Benoit, Assenmacher, & Walter 1950 ; Greeley & Meyer 1953 ; Lofts & Marshall, 1958). Most workers suggest that it is the anterior pituitary that is the seat of seasonal negativity. There is no anatomical evidence that this is so—yet there is both experimental and histological evidence that the adenohypophysis is at least involved. The testes of Arctic and desert non-breeders sometimes metamorphose before they reach full spermatogenesis. That is probably due to effects, exerted by the unsatisfactory state of the environment, on the central nervous system and, it follows, on the anterior lobe which then ceases to produce gonadotrophins (Marshall 1952a).

The evidence customarily used to implicate the anterior lobe could apply equally well to the hypothalamus, or any other part of the central nervous system that periodically activates the adenohypophysis. We know that single-brooded species regularly become " refractory " after one nesting, and plural-brooded birds only after several, and this may suggest that the factors controlling the onset of this regenerative period of sexual negativity are neural ones. Ultimately these may be dictated by external events including, particularly, behavioural reactions between the pair.

During the regeneration phase sexual behaviour is nil, or almost so. Many normally solitary species tend to flock, often in company with other species. The post-nuptial moult begins, and proceeds strongly. The duration of the period in the wild bird appears to be stable within the species. As a result of photostimulation work, it can now be predicted when a given spring-breeding species will end its regeneration phase, i.e. cease to be " refractory ". Most North temperate zone species end their regeneration period between August and November. Some believe that a period of short days or long nights of winter are needed before the regeneration period will end (Burger, 1947, 1949 ; Farner, 1955, Wolfson, 1959 a, b). We will see that in many species no such influence is necessary.

Acceleration phase. This follows the regeneration phase automatically. The bird has now reached a physiological condition in which its neuro-endocrine apparatus is susceptible to external stimuli. Morley (1943) and Marshall (1952 b) have provided numerous references that many British birds, and others elsewhere, undergo autumnal sexuality. This follows the renewed secretion of gonadotrophins which, in turn, reactivate the gonads and cause the flow of sex hormones, the sharp or gradual disappearance of the last remnants of tubule lipids and the occasional autumnal multiplication of germ cells. This phase varies considerably in its duration between species as well as in relation to the state of the environment, including the partner and the flock. In most birds the post-nuptial moult ends early in this phase. Among Arctic and desert non-breeders, as well as birds in zoos, progression is halted by environmental influences (Marshall 1952 a, Keast & Marshall 1954). The acceleration phase is a period of territory selection, song and increasingly intensive sexual activity. Some species develop secondary sexual characters (e.g. the assumption of beak colours and nuptial plumes). An early start of such activities (in autumn) is probably conducive to later reproductive success. At the same time, external inhibitors (e.g. cold) will temporarily halt the process late in autumn and prevent the wastage of the reproductive potential in winter. Among many tropical species the advent of dry weather, or otherwise unfavourable external conditions, does likewise. Among other tropical species, e.g. Ascension Island sooty tern, which breeds four times every three years (Chapin 1954), and certain other birds (see Miller 1954, 1959, Marshall & Roberts 1959) the absence or relative absence of such inhibitors permits the abandonment of the more or less precisely annual cycle. As other investigators turn their attention to different parts of the world, more and more species will be found to exhibit more than one complete cycle each year.

In temperate-zone birds whose acceleration phase began in late summer or early autumn, and was depressed or halted in winter, there is after the winter solstice a sharp, probably photostimulated, renewal of sexual behaviour, accompanied by gametogenesis. The cycle now continues under the influence of spring stimuli and inhibitors until its culmination with reproduction.

Culmination phase. Now nest building is completed and insemination and ovulation occur. A special *species requirement* of end-stimulation is required before the cycle culminates in reproduction. Towards the end of the acceleration phase the testes contain massive bunches of spermatozoa. Many

are free in the lumina and great numbers have already reached the seminal vesicles. The female, whose accessory sexual organs have been enlarging but which has nevertheless lagged behing the male in her gametogenetic development, is ready to begin the final swift oocyte development that will end in ovulation and egg deposition through a now grossly hypertrophied oviduct. But although the internal physiology may be now wholly prepared for reproduction, this phase, like that of acceleration, is highly susceptible to stimuli (e.g. that of the nest, behavioural interactions etc.) and inhibitors (e.g. sudden cold, fear or nest-destruction). In many, perhaps most, species it is the end-stimuli that permit nidification, insemination and ovulation which are the real " timers " of the sexual cycle. There is no evidence that the current photoperiod has any influence on it whatever (Marshall, 1949 a). The period lasts until the final clutch is laid, after which individuals once more enter the phase of sexual negativity and regeneration described above.

INTERNAL RHYTHM IN ASEASONAL BREEDERS

Great numbers of species both within the tropics and outside them do not breed according to any particular phase of the cycle of the sun. It is in such species that we see most clearly the operation of an internal rhythm of reproduction. The supreme virtue of light as a regulator is its regularity ; but likewise (a point which cannot be over-emphasised) this unvarying regularity must be a force of destruction to any animal compelled to obey it in a habitat in which good conditions for the survival of the young did not accompany a particular phase of periodicity. It is a condition of their survival that many species reproduce at times when rainfall has rendered the environment propitious for the development of their offspring. If the sexual cycle of many xerophilous (not necessarily desert) species was governed by photoperiodicity the young would be launched, more often than not, into arid conditions which would not allow them to survive.

We have seen above that at least some such species have developed a mechanism which allows them to become sexually mature at the age of sixty days. Thus, budgerygahs and zebra finches will produce spermatozoa at that age even when kept in almost total darkness provided they are given adequate warmth, food and water. In aviaries both the above-mentioned species will reproduce almost all the year round under optimum conditions. It is an engrossing thought that the mechanism evolved by budgerygahs around Oodnadatta is the same that permits the species to breed the year round in captivity at Stoke Poges. This aspect of drought adaptation is one of the factors that has allowed the budgerygah to become perhaps the commonest bird in both these rather different environments. In the dryer areas of Australia their vast flocks undulate over the horizon and are responsible for the specific name *undulatus*.

We have dealt in detail with only two well-known species but Serventy & Marshall (1957) have indicated elsewhere that the effects of chance and other rainfall, coupled with temperature, are the essential breeding regulators of a great diversity of species over wide areas of Western Australia. It is obvious that not only physiological, but also behavioural adaptations must be involved

in the sexual cycles of many such birds. In particular, display must be abbreviated.

In the temperate zone satin bowerbird (*Ptilonorhynchus violaceus*) of the lush forests of Eastern Australia the flashing, staccato display—which, incidentally, strongly resembles that of a Spanish flamenco dancer—may continue for up to four months (Marshall, 1954). In temperate zone species which live in dry areas of inland Australia such prolonged splendour is impossible : species leap from sexual inactivity to full productivity in a matter of days. They must conclude their display, reach reproductive condition and produce their young while the beneficial rain-induced conditions last in their precarious environment. Thus, McGilp (1919) made the extraordinary observation that the desert bushchat (*Ashbia lovensis*) began nesting five days after the beginning of a two-day downpour which broke a prolonged South Australian drought. It laid eggs eleven days after the beginning of the rain.

The desert bushchat mentioned above, and many of the species investigated by Keast & Marshall (1954), inhabit periodically drought-stricken semi-desert and depend for reproductive conditions on chance rainfall. There is also a large category of species that live in *relatively* dry areas which receive one or sometimes two rainfalls every year. While travelling in the Kimberley region of north-western Australia recently, Serventy and I (unpublished) found that long before the fall of expected rains, the sexual cycle of some birds was actively in progress. In such areas the advent of the expected rainfall would bring the cycle to fruition. When, as frequently happens, rain does not fall, the cycle is inhibited (Robinson, 1933 ; Marshall, 1951 a, Carnaby, 1954).

The grey teal (*Anas gibberifrons*) of Australia is an arresting example of a species that has been extremely successful in its behavioural and physiological adjustments to a capricious climate. Dependent over great areas of its range on temporary water-holes filled by chance rainfall, the grey teal has abandoned an annual cycle. Furthermore, it has reduced to a minimum the long courtship period characteristic of the genus *Anas* (H. J. Frith, personal communication). By nomadic movements it finds water-filled clay-pans and billabongs where its sexual cycle quickly reacts to produce young while the water, and, it follows, food lasts. Remarkably, the grey teal has become the most successful Australian duck.

Australia has long enjoyed a curious reputation for peculiar phenomena—" songless bright birds " (an absurd European belief), trees that periodically shed their bark instead of leaves, mammals that lay eggs and so on. There is however, nothing mystical about the breeding seasons of Australian birds, and to emphasize this I will turn to another great land mass, Africa.

Here, many species—both aquatic and land birds—have likewise adjusted their physiology and behaviour to take advantage of propitious conditions whenever they occur. For example, the small xerophilous dioch or red-billed weaver-finch *Quelea quelea* has mastered its arid environment and become one of the commonest birds in the land. This species is so numerous in vast areas of Africa that it reaches plague proportions and today breeding colonies are systematically blown-up in an attempt to bring the pest under control.

Although *Q. quelea* retains an ancestral capacity for photostimulation (Marshall & Disney, 1956), such is not influential under natural conditions. If it were, the species (the adults of which can subsist on only seeds) would reproduce at periods during which the environment lacked the materials traditionally used for nesting and the amino-acids essential for the maturation of their young (Marshall, 1951 b ; Disney & Marshall, 1956).

The reproductive cycle of *Q. quelea* has been experimentally studied by Disney, Lofts & Marshall (1959) in order to determine the length of the regeneration (refractory) period. We have seen that in the various spring-breeding temperate zone species so far investigated this period lasts for several months. For how long does this temporary period of enforced sterility last (if it exists at all) in the reproductive rhythm of a species in which successful reproduction can occur only after rainfall that produces conditions propitious for breeding and the survival of young?

It was found that a post-nuptial period of sexual regeneration does in fact exist, but that it is relatively brief. Thus, after each breeding season the males, at least, progress with little delay into a condition whereby they can take advantage of propitious breeding conditions whenever these arise in the arid environment to which they, and especially their reproductive processes, are adapted. Again we see a rapid completion of courtship activity. Within a few days of the arrival of the horde on the nest-site, each male starts to build and to display vigorously in the incomplete structure. This attracts a female and copulation has been observed in and alongside the nest (Haylock, 1954 ; Morel & Bourlière, 1955 ; Morel & Morel, 1955).

Numerous other species in many parts of the world will be found to behave like those dealt with above. I see no reason why rain-induced reproduction should not be discovered in the south-western United States and Mexico. Certain aquatic birds on the Victoria Nyanza (Marshall & Roberts, 1959), finches of the genus *Munia* in Borneo (Marshall & Harrisson, unpublished), have abandoned an annual cycle. Miller (1959) has carried out important work in Colombia by the use of rings and has shown that *Zonotrichia capensis* can reproduce at any time, and that individuals breed rhythmically twice in a single year.

We have seen that both physiology and behaviour—in fact, the entire *biounit*—can change according to the environment to which the animal adapts itself. We have seen further the remarkable phenomenon of creatures mastering harsh unpromising conditions to such a degree that they achieve a greater biomass than closely related neighbours enjoying exceedingly lush conditions.

At the same time, it has to be confessed that we know little of the details involved. As mentioned before, there is evidence that a species can ovulate within eleven days of the long-delayed fall of rain. It follows that, in those species that require rain-induced cover in which quickly to nest, the preliminary stimulus should be rain itself. As yet there appears to be only one piece of evidence that this is the case. Marshall & Disney (1957) designed an experiment that showed that first-year *Q. quelea* were influenced by the green grass that grew after rainfall in tropical Tanganyika. When the experiment was repeated with *older* birds it was found that rainfall itself appeared to exert a strong

stimulus to building. That rainfall (or humidity) affects the neuro-endocrine system was indicated by old non-breeders which moulted from one breeding dress to another, without the customary intervening period of neutral plumage.

We know that once the lengthy regeneration period has ended in some species, increased light can stimulate the sexual cycle (including behaviour) which then comes under the control of various stimulating environmental factors (of which temperature is of almost universal importance) significant to the particular species. It seems almost certain that, in numerous other species, once the abbreviated regenerated period has ended, rainfall *per se* can stimulate the sexual cycle which then likewise comes under the control of a variety of significant external factors. Each kind of bird has its own innate " species requirement ". The individual components of this complex will have to be determined species by species.

INTERNAL RHYTHM IN TRANS-EQUATORIAL MIGRANTS

We have seen that the post-nuptial period of regeneration has been foreshortened in species for which it is beneficial that reproduction should occur at any time of the year. I believe that this endogenous component of the cycle is adaptive in the other direction and that a lengthening of the period of sexual negativity may be the principal timing factor in equatorial and trans-equatorial migrants which begin their nuptial journey under almost constant, or decreasing, daylengths. For example, some populations of yellow wagtails (*Motacilla flava*) " winter " on the equator (or south of it) and arrive in their European breeding grounds with considerable regularity each northern spring. It seems possible that, after the automatic emergence from a prolonged regeneration period, involving sex-hormone liberation, *various* environmental factors (including particularly behavioural inter-actions) start the cycle anew. Thus, the behaviour of yellow wagtails " wintering " on the equator begins to change some time before migration. With the first manifestation of gametogenetic development in each sex, a greater social cohesion becomes apparent and the birds begin to associate in pairs, no doubt as a result of the post-regeneration production of sex hormones. After a further short period of almost constant day-length the birds leave for their northern nuptial grounds where their breeding season (and sexual cycle) is probably ultimately timed by the external factors that permit successful nesting and reproduction (Marshall 1951 a, 1955 ; Curry-Lindahl, 1958 ; Marshall & Williams 1959). Laurence Irving (personal communication) has evidence that *M. flava*, arrives in Alaska (for example) with considerably enlarged testes. Eggs have been recorded within ten days of the arrival of the birds on the breeding ground.

That an internal rhythm of reproduction does in fact, exist in one trans-equatorial migrant, the short-tailed shearwater (*Puffinus tenuirostris*), has been experimentally proved by Marshall & Serventy (1959). This species undertakes annually a mighty circum-Pacific journey from Southern Australia to Japan, the Aleutians, Western North America and back across the Pacific to Australia with astonishing regularity. It returns to its breeding island during the same twelve days every year (Serventy, 1953). Marshall & Serventy (1956) showed that the migratory flocks of this " mutton-bird " regularly

leave Southern Australia in April (autumn) at a time when their principal food—euphausiid plankton—remains abundant, and when sea temperatures are still higher than at the November period of ovulation. It was concluded that the post-nuptial northern journey via Japan to the Aleutian and South Arctic contra-nuptial (" wintering ") areas is probably in obedience to an inherent internal rhythm. When the millions of birds return and first make their landfall at the breeding islands in the last week in September (spring), gametogenesis is already well advanced. The testes contain bunched spermatozoa. There is no doubt that both the spring sexual cycle and the southern journey are initiated under conditions of decreasing day-length whilst the birds are still north of the equator. However, the astonishing regularity of their Australian land-fall and subsequent egg-laying (19th to 21st November and the following twelve days) suggests that the nuptial journey must be " timed " by some astronomical constant, possibly decreasing daylengths, as appears to be the sexual cycle of certain fish (Hoover & Hubbard, 1937) and mammals (Yeates, 1949). No obvious periodic changes that might operate as precise " timing " factors were observed in the breeding area.

By varying the light regimes of experimental captives it was found that decreasing daylength seems to have no effect whatever. Both experimental and control birds came into breeding condition at approximately the same period as the wild population on the completion of their annual journey. Thus, the breeding rhythm is persistent at least for one year after the animal is taken out of its normal environment and exposed to external influences (photoperiod, temperature, food and habitat) exceedingly different from normal. It must be emphasised, nevertheless, that although gametogenesis and premigratory fat deposition took place under these conditions at approximately the normal periods, there was in some individuals a considerable asymmetry of the seminiferous tubules and a pronounced retardation of spermatogenesis that is never encountered in the wild population. This showed that internal events were tending to drift out of phase with the external environment and that as yet undetermined external influences, possibly including behaviour, were lacking. In short, although an internal rhythm indubitably exists, it must be regulated annually if it is to remain appropriately synchronised with the seasons.

DISCUSSION

It should be emphasised that the statements set out above perhaps represent only the opinions of myself and a group of people who have worked in my laboratory and collaborated with us in studies carried out elsewhere. It should be pointed out, too, that the more " orthodox " views are those held by my friends Farner (1955) and Wolfson (1959 a, b) (and others) who ascribe a far greater general importance to photostimulation than do we. Wolfson in particular argues that, even on the equator, the migration and reproduction of birds are under photoperiodic control.

We believe that a great number of species, even those existing in the temperate zones of considerable photo-fluctuation, are uninfluenced by photoperiodicity and that were they so influenced they would be much less successful and, in many instances, would have probably disappeared by the agency of

natural elimination. The importance of a partly endogenous, partly exogenous internal rhythm of reproduction has been stressed, and it is suggested that the stable endogenous component is adaptive and has been varied in its duration from species to species according to habit and environment. Where the environment is so fruitful that the young of more than one season a year are supportable (e.g. the Victoria Nyanza in the case of cormorants), or where the environment is generally so unproductive that it is essential that reproduction should occur whenever rain falls (see below), the length of the post-nuptial regeneration period is often shortened by natural selection. The length of this period, too, is possibly influential in the timing of transequatorial migration.

The more prolonged exogenous component is, of course, modifiable by week to week events in the environment such as light (in many species), temperature (in almost all non-tropical species), danger, food and water supply, nest-site availability, and behavioural reactions between the sexes. It is only when we study reproductive processes by the use of changing (and, whenever possible, unstable) environments (ideally over a succession of years) as a kind of natural laboratory that we see clearly the operation of factors such as those mentioned above.

Even in climatically stable areas the mean reproduction date of a sedentary species may vary by as much as a month from one year to another. It is believed that in most species, whether the post-regeneration cycle is initiated by light or not, the sexual cycle is " timed " by external stimuli presented by the traditional breeding ground to the female exteroceptor organs. In all species yet investigated the males quickly achieve gametogenesis, far in advance of the female. Not until the environment presents the " species requirement " that she innately requires will the female's cycle culminate and make reproduction possible.

The sexual cycles of some species appear to be accelerated by rainfall, or by its effects (such as the growth of herbage, or the rise of water-level in lagoons) and, of course, by the increase (but not, in birds, so far as is yet known, by the decrease) of daylength. The advantages of such mechanisms have been outlined. A consideration of these *various* complexes reveals clearly how completely the *biounit*—the whole animal, not merely the sexual cycle—is involved. Thus, for example, the widely unrelated finch *Quelea quelea* and duck *Anas gibberifrons*, which breed swiftly and opportunistically as soon as chance rainfall renders their unpromising environments propitious for reproduction, have correspondingly abbreviated their sexual displays, the latter in startling degree by comparison with other Anatinidae.

If we consider all the implications of, for example, innate nomadism and traditional species requirement of the female on the breeding ground, we will see that neural adaptations too, varying between closely related species and perhaps races, have been dictated by the environment. The interplay of a variety of factors has enabled *A. gibberifrons* probably to become the commonest duck in Australia and *Q. quelea*, in terms of biomass, perhaps the most successful finch in Africa. The latter is such a plague-pest that the Federation of Rhodesia and Nyasaland, the French Republic and Community, Belgium, Ghana, Guinea,

Liberia, Portugal, the Union of South Africa and the United Kingdom all contribute funds to the *Commission de Coopération Technique en Afrique au Sud du Sahara* to be used for its destruction. In their millions, the highly nomadic adult finches can subsist essentially on dry seeds during the arid season and, having quickly passed through the regeneration phase, rapidly reproduce during any subsequent period of the year in which rain falls and causes the environment to present optimum amounts of nest material and, it follows, associated proteinous insect food on which the young subsist for the first five days of life.

On the other hand, many species of the same environment remain relatively unsuccessful and some, no doubt, have disappeared altogether.

The day is almost here when the study of avian breeding seasons will cease to be an endocrinological problem : it is even now essentially the business of the ecologist, ethologist and neurophysiologist.

SUMMARY

1. There exists in seasonal birds a partly endogenous, partly exogenous, rhythm of reproduction involving successive phases of post-nuptial *regeneration*, *acceleration* and *culmination* which can be considered as a cogwheel which is engaged at various times by changing environmental " teeth " that are helpful, or otherwise, to reproductive success and therefore to the existence of the species.

2. The duration of the regeneration phase is adaptive and, depending on its length, this phase may be an important internal " timing " device in migration and reproduction. Thus, some species have developed an extremely abbreviated, and some a lengthy, such period according to the productivity and exigencies of their environment.

3. After the spontaneous emergence from the regeneration phase (which seems to be constant in its duration within the species) into the acceleration phase (when sex hormones are again liberated and spasmodic display occurs) the neuro-endocrine machinery of each individual comes under the week-to-week influence of two antagonistic sets of external factors, i.e. *accelerators* and *inhibitors*.

4. Light is merely one of the significant post-regeneration accelerators of the sexual cycle : light is important only in species for which it is important that it should be important. Other significant accelerators are warmth, territory, adequate food, nest site, nesting materials and behavioural inter-actions. Inhibitors are cold, inclement weather, hunger, fear and lack of appropriate nesting material and the traditional nest-site. This is particularly true of the female.

5. External *culminators* (which are often also accelerators) bring the sexual cycle to a successful conclusion. No single over-all factor (such as day-length) is responsible for the timing of avian breeding seasons. The breeding season is kept in step with the sun essentially by the external factors that influence the female exteroceptor organs and ultimately permit nidification and ovulation on the habitual breeding ground. Of these, the most important may be appropriate nesting materials, a safe nesting site, the nest, mild weather and an

abundance of the food on which the young are traditionally fed. Until the environment presents the pair with its *species requirements* the mutual end-behaviour essential to reproduction will not occur.

6. The *biounit*—the whole animal—is involved in any successful adjustment of the sexual cycle to external conditions. Thus, in some species, the post-nuptial period of regeneration is remarkably shortened and an innate neuro-endocrine display is elicited by the spasmodic experience of rain (or tall grass or rising water in lagoons). Further, the lengthy period of display, traditional to the type, may be abbreviated dramatically with the result that preliminary sexual processes are speedily concluded, and reproduction occurs, while the environment still retains near-optimum conditions for the survival of the young.

7. By the singular perfection of such adjustments some species are able to prosper in relatively harsh and apparently unpromising conditions to a degree whereby they achieve a far greater success than do close allies that are confined to lush regions nearby.

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ADDENDUM

Frith has now published highly significant information on the differential breeding ecology of three species of Australian ducks. Sexual activity in both grey teal (*Anas gibberifrons*) and the pink-eared duck (*Malacorhynchus membranaceus*), which exploit temporary floodwaters, seems to be stimulated by increasing water-level. In the former species (see p. 62) actual reproduction occurs quickly and coincides with the rising of the waters whereas in *M. membranaceus* breeding is delayed until their decline. As a result, both species breed at any time of the year, irrespective of the cycle of the sun, yet the emergence of the ducklings of each coincides with an increase of animal food appropriate to the species (e.g. abundant plankton in the case of the later-breeding pink-eared duck).

The black duck (*Anas superciliosus*), on the other hand, exploits chiefly the more permanent waters and it is far less sensitive to changes in water-level. It has a more regular (spring) breeding season and some birds reproduce even when environmental conditions are adverse ; nevertheless, some out-of-season breeding takes place whenever exceptionally favourable conditions occur.

For anybody familiar with the prolonged social and sexual display of the Nearctic and Palaearctic mallard (*Anas platyrhynchos*) Frith's data on the Australian *A. gibberifrons* must be of exceptional interest. In this opportunist breeder the whole process of display, and the prospecting of a nest site (in a hollow tree), may take a mere ten to seventeen days. The Australian *A. superciliosus*, on the other hand, has prolonged autumn and spring social and pairing displays not unlike those of the mallard.

We may expect data comparable with the above from other continents in the future.

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SOME PROBLEMS OF ADENOHYPOPHYSIAL RELATIONSHIPS IN CYCLOSTOMES AND FISH

BY

E. J. W. BARRINGTON

Department of Zoology, Nottingham University

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INTRODUCTION

This symposium is strikingly demonstrating the complexity of the problems involved in the analysis of endocrine cycles. To take only one example, the activity of the thyroid gland clearly shows some degree of cyclical variation, but Matty and Swift have well demonstrated the difficulty of precisely defining the form of the cycle, and have suggested more than one function to which it might be related. The more that one considers this situation, the more remote appears any prospect of being able to establish a simple one-to-one relationship between the activity of a particular gland and some individual aspect of an animal's life. In the simplest case we might, perhaps, conceive of an endocrine gland as exhibiting a purely autonomous rhythm (see below) and influencing, through this, some other gland or some other function. Or we might conceive of a non-endocrine rhythm affecting an endocrine gland by making a cyclical demand upon its secretory product and thereby establishing a cyclical rhythm in its activities. Or again, we might conceive of an endocrine rhythm as being primarily determined by cyclical change in the external environment, as Swift (this symposium) suggests for the annual thyroid cycle of immature trout, or as being the resultant of interacting forces, in part exogenous and in part endogenous, as apparently in the spermatogenetic cycle in Amphibia (van Oordt, this symposium) and in the breeding cycle of birds (Marshall, this symposium). With so little known at present about the mode of action of hormones, we can hardly hope to unravel such causal relationships with any great precision, nor, indeed, as Marshall rightly points out, is this a purely endocrinological problem. However, I should like to take this opportunity of indicating the nature of some of the relevant problems as they have presented themselves to us in essentially endocrinological studies in my laboratory, and to suggest some types of information, particularly in relation to the functioning of the adenohypophysis, which might eventually contribute to a solution of them.

One problem which has attracted our attention is the possible influence of endocrine secretions upon growth in the lower vertebrates. Now it is known from the work of Brown (1946) that if fish are kept in the laboratory under constant and carefully controlled conditions their growth does not necessarily take place at a constant rate. She maintained two-year old brown trout, *Salmo trutta*, at a constant temperature and with a constant rate of water flow and with twelve hours per day of artificial illumination, and provided them with an *ad libitum* supply of food at regular intervals. Such fish showed marked variations in their specific growth rate during the year, with maximum rates from February to May and minimum rates in October and November. Under hatchery conditions the pattern of variation is more complex (Brown, 1957 ; Swift, 1955), as would be expected, one factor being that temperature will affect growth rate by virtue of its influence upon the appetites and maintenance requirements of the fish. Nevertheless, Swift was able to demonstrate that in three-year old brown trout there was a marked increase in growth during early spring, leading to a maximum towards the end of April, and that there was a decline during autumn to a low winter level. According to his analysis this cycle, which clearly showed a fundamental similarity to that demonstrated by Brown in the laboratory, could not have been influenced solely by temperature. He suggested, however, that the acceleration of growth in the spring might be associated with increasing day length, and that this might be mediated by pituitary growth hormone. Such an interpretation would seem to carry the implication that there must be an endogenous cycle in the production of this hormone, modulated, perhaps, by the external environment, but capable of manifesting itself under artificially controlled and constant conditions in the laboratory.

That pituitary growth hormone can indeed affect the growth of teleost fish has been clearly established by Pickford (1953a ; 1953b) in her studies on the male killifish, *Fundulus heteroclitus*. She has shown that growth in length of this animal ceases after hypophysectomy, that weight changes are irregular, that no new circuli are added to the scales and that no further material is deposited on the otoliths. These effects can be reversed by injections of purified crystalline beef growth hormone, the results being the more striking in that control saline injections produce shock effects which cause at first some loss of weight ; in consequence of this, the influence of the growth hormone is not statistically significant until the end of the third week of the injections.

From all of these results we might well feel justified in suspecting that an endogenous cycle in the production of pituitary growth hormone could be a relevant factor in the establishment of growth cycles in teleosts, but there was an interesting suggestion in Pickford's data that the pituitary might not be the only endocrine gland involved. In one of her experiments (1954a) she injected one group of her hypophysectomized fish with fish growth hormone and a second group with beef hormone, and found that while there was a stimulation of growth in weight and length in both groups, the effect was significantly weaker in the former. A possible causal factor here was the known presence in the beef preparation of sufficient thyrotrophic hormone to maintain the thyroid at a normal level of activity, as judged by its histological appearance, while

this appeared to be absent from the fish preparation, those animals receiving the latter having at the end of the experiment a completely inactive gland. In a further publication Pickford (1954b) was inclined to attribute this effect to the influence of thyroid hormone on general health rather than to any specific growth effect, but her interesting results clearly suggest that the response of *Fundulus* to pituitary growth hormone may depend upon the degree of activity of the thyroid gland, and that the possibility of the latter having a direct effect upon growth cannot be entirely rejected.

In this connection it is significant that we have been able to show at Nottingham, in still unpublished work, that treatment of trout with thyroxine produces a very striking stimulation of growth both in length and in weight. In one experiment, for example, yearling rainbow trout (*Salmo gairdneri*) were immersed in a 1:500,000 solution of thyroxine in a constant temperature room and the progress of individual fish compared with untreated control animals. Data collected by Dr D. H. Piggins from this experiment showed that in the course of five weeks the mean length of twelve thyroxine-treated fish had increased by 21·7 per cent as compared with 8·3 per cent for a similar group of control fish, the corresponding weight increases being 98·3 per cent and 40·7 per cent, these differences being statistically significant. Fontaine & Baraduc (1955), in experiments carried out at the same time as ours, and independently of them, have also reported that the addition of thyroxine to the diet of rainbow trout can under some circumstances promote growth in weight. Our own work is still in progress, since we are particularly interested in the possible influence of thyroid hormone upon nitrogen metabolism. It is not yet possible, therefore, to estimate the significance of this growth effect in the normal life cycle, nor, if it is significant, can we judge whether the thyroid hormone is acting independently of the growth hormone or whether the two are integrated by some common regulating factor, acting perhaps at hypophysial level. However, we might well feel justified at least in looking for some correlation between thyroid cycles and growth cycles in the teleosts, yet in fact we learn from the analysis of Swift (1955) that in three-year old brown trout there is a peak in thyroid activity in midsummer at about the time when, under hatchery conditions (in contrast to the laboratory conditions under which Brown's studies were carried out), there is a sharp decline in the growth rate prior to an increase to another peak in August. Swift, however, has suggested that this decline is due to a shortage of metabolites, and that the actual correlation here may be between the activity of the fish or of its reproductive system, and the activity of the thyroid gland.

Now Hoar *et al.* (1955) have presented evidence that both testosterone and thyroxine promote increased activity in goldfish and in young salmon, and it seemed to us worth while to examine how far similar influences might bear on the activity of the minnow, a fish to which we have devoted a good deal of attention. Seasonal variation in the activity of this animal is suggested by some observations of Woodhead (1956), who found that the mean swimming speed of pre-spawning minnows in mid-April was 159 per cent faster than that of fish studied in December, even though they had been kept in aquaria for at least seven months prior to their examination. Confirmation and amplification

of this has been obtained at Nottingham as a result of careful and painstaking studies carried out by Dr Aminul Haque, the principle of the method which we devised being to place the animals in an observation trough through which currents of water could be passed and then to record their periodical excursions " upstream ". Haque was able to show that there was a well-marked cycle of activity both in fish which were kept in outdoor tanks and those which were being maintained indoors in aquaria. From February onwards the activity rose to a peak in July, following which there was a steady decline leading through a sharp fall in November to minimum activity in January. Now there is evidence from work both at Nottingham (Barrington & Matty, 1954) and elsewhere that an annual cycle of thyroid activity can be detected in the minnow, and this has already been discussed by Swift and Matty in this symposium. The exact shape of the curve is uncertain, Fortune (1955) having reported a peak in late summer in addition to one in April and May, so that it is difficult to see whether it is correlated with the activity cycle, but it seemed worthwhile to us to examine how far hormonal treatment might affect the swimming of the minnow. It is not possible to discuss this work here, but it can be said that the results of thyroxine treatment were substantially negative for most of the year. This may not mean very much, for such experiments are time-consuming and it is impossible to vary the conditions of them as much as one would wish, so that negative results may merely reflect a failure to achieve the correct degree of physiological stimulation. At this stage, then, one would merely conclude that in any investigation of thyroid cycles we must take into account the possibility that they may be related to activity cycles, and that even when the shape of a thyroid activity curve is clearly defined, its interpretation may be very uncertain until we have some knowledge of the interplay of the factors which determine the form of such curves. Thus the fact that hatchery trout show a decline in growth rate at a time when thyroid activity is maximal does not necessarily dispose of the possibility that thyroid hormone is of physiological significance in the growth processes of fish ; it could mean no more than that at that particular time some other factor (e.g. activity, according to Swift's analysis) may have an over-riding influence.

The situation is further complicated by the undoubted fact that many physiological cycles are integrated with the external environment, a phenomenon which, as we have already seen in this symposium, is particularly well-established in the case of reproduction. Thus we know that while minnows normally come into full reproductive condition during May, they will do so earlier if the animals are subjected to additional illumination (Bullough, 1940a). We also know, however, that sexual maturation is not abolished if they are placed in continuous darkness during the early months of the year, although it may be somewhat delayed (Bullough 1940b), and this suggests that here, as also in birds, there may be an endogenous rhythm which acts in the absence of external seasonal changes but is normally reinforced and precisely timed by them. A similar suggestion emerges from Brown's work on growth, for she found that even when trout had been reared under constant laboratory conditions for more than one year they underwent sexual maturation at the normal time towards the end of the third year of their life (Brown, 1957).

Now an annual reproductive crisis, with its associated spawning behaviour, may well be expected to involve other hormones than the sex hormones in the restricted sense of that term, and we have long felt that there is good reason for suspecting that the thyroid hormone may be involved in teleosts. Matty has already developed this point in this symposium, and I need only add here that in the course of Dr Haque's work it emerged that treatment of minnows with thyroid hormone evoked the increased deposition of guanine at the base of the paired fins and the development of tubercles on the head. Both of these are well-recognized secondary sexual characters, which are produced also by testosterone treatment, and in view of what has been said above on the possible relation between thyroid secretion and locomotor activity it is of interest that Woodhead (1956) found guanine spots well-developed in his group of minnows which showed high swimming speed in mid-April. Here then is certainly a suggestion that the thyroid hormone may be involved in the reproductive cycle, and, of course, another possible link between this gland and nitrogen metabolism.

Marshall has suggested here that the study of breeding cycles in birds may soon cease to be an endocrinological problem, and it is certainly true that endocrine cycles demand to be studied within the widest possible field of reference. I myself draw from this discussion, and from the papers in this symposium, the conclusion that we must be prepared to extend our investigations to include the endogenous factors by which these cycles are determined, the paths by which these in their turn are brought into relationship with the external environment, and the means, if any, by which the several endocrine organs may interact with each other with consequent synergistic or antagonistic effects. In such studies the pituitary gland must be of fundamental importance, for it is well-established for higher vertebrates that the activities of the reproductive system and of the thyroid gland, for example, are regulated respectively through the feed-back systems associated with the gonadotrophic and thyrotrophic cells of the adenohypophysis, while Harris has surveyed here today the fascinating problems involved in the analysis of the relationships of the latter with the hypothalamus and, through that, with the outside world. What I propose to do now is to consider some of these problems of adenohypophysial relationships in the lower vertebrates, viewing these against the general background which I have been trying to outline.

CYCLOSTOMATA

It is an obvious obligation of the comparative endocrinologist to enquire into the evolution of the systems with which he has to deal, not only because of the intrinsic interest of the story which emerges, but because it is likely to contain the key to many problems of the organisation and functioning of these systems in the higher forms, and I should like to comment from this point of view on some aspects of the pituitary-thyroid relationship. As regards thyroidal biosynthesis, recent investigations have established that the iodination of tyrosine occurs in a wide range of invertebrates (Gorbman, 1955), possibly as a by-product of their organization and without being of any functional significance for them, although this rather negative interpretation has as yet

no experimental evidence in its support. Even in the chordates, thyroidal activity precedes the development of the thyroid gland, for thyroidal biosynthesis is detectable in the endostyle of *Amphioxus* and the ascidians (Barrington, 1959). It is there associated with a distinctive group of cells in a manner which suggests that its existence is biochemically purposive rather than merely random, and that the iodinated products are therefore of some functional significance to the animals concerned. At this stage, however, there is no pituitary gland present, for the most likely homologue of this organ would seem to be Hatschek's pit in *Amphioxus*, and, although the function of this structure is not understood, there is very little indeed to suggest that it could be functioning in a manner at all closely comparable with either an adenohypophysis or a neurohypophysis. If, therefore, there is any regulation of thyroidal biosynthesis in these groups, it must presumably depend on autonomous responses of the secreting cells themselves, but in the absence of any relevant evidence it would be futile to consider this aspect any further.

When we pass to the Cyclostomata we are dealing with vertebrates which, although at a level of organisation substantially lower than that of fish, nevertheless possess thyroid and pituitary glands which are recognizably comparable with those of higher forms, and it is of importance in tracing the evolution of vertebrate endocrine cycles to determine the degree of relationship existing between these organs at this stage.

Let us first see what conclusions can be drawn from the organisation of the pituitary itself. It is easy to identify an adenohypophysis and neurohypophysis, the former developing from the solid nasohyophyseal stalk and the latter appearing as the floor of the infundibular cavity. The adenohypophysis is differentiated into three regions which I shall here refer to as the anterior, median and posterior zones. As regards the anterior zone, we agree with other observers in finding in it numerous cells with contents staining with aniline blue and giving a positive PAS reaction, and there is a general tendency to interpret these as gonadotrophs (Lanzing, 1959 ; Kamer & Schreurs, 1959). It has, however, been briefly reported by Roth (1958) that in their histochemical reactions these cyanophils show certain properties which characterize the acidophils of mammals, and he has suggested that this single cell type may combine functions which in the latter group are distributed between several distinctive types. Granted that we have singularly little basis for any estimate of the significance of such tests, his suggestion needs to be borne in mind in any histological analysis.

Evidence as to the existence of thyrotroph cells in the pituitary of the lamprey is at present much less certain. By analogy with higher vertebrates we should expect such cells to be cyanophilic, but there appears to be no indication of the presence of a second type of cyanophil in the anterior zone. In the median zone the most conspicuous cells are chromophobes, but scattered amongst them are a much smaller number of irregular, triangular or polyhedral cyanophils, and it has been suggested (Lanzing, 1959 ; Kamer & Schreurs, 1959) that the latter might be thyrotrophs. We are at present conducting experiments to test this hypothesis, which as yet has little direct evidence in support of it, although Kamer & Schreurs attach importance to the fact that these cells become active (as determined by the presence of secretion) shortly before metamor-

phosis and reach a peak of activity during metamorphosis. It is, however, difficult to discover from the context exactly how far the period of metamorphosis is regarded as extending in this interpretation, and it needs to be remembered that thyroid hormone has never yet been shown to influence this process in the ammocoete larva. As regards the aldehyde fuchsin stain, which can be used to differentiate thyrotrophs in the minnow (Barrington & Matty, 1955), as well as in mammals, we have observed that it colours the cyanophils of the anterior zone and also the scattered ones in the median zone. The procedure, however, is a purely empirical one, many diverse structures in a section may be coloured by it, and its analytical value depends on critical consideration of all of the relevant circumstances. As far as the cyclostomes are concerned, this value is at present non-existent.

More could be said on this problem of the organization of the lamprey's pituitary, but for the present we can conclude that its degree of differentiation in the adult does not exclude the possibility that it secretes a thyrotrophic hormone, but that convincing evidence for this is only likely to emerge from suitable experimental treatment. If this is as far as we can go in the adult, the situation in the larva is even less clear, for while in the former the main features of the organization of the pituitary are well seen, the cytological pattern in the ammocoete is much less clearly defined and in some respects only becomes expressed in a well-differentiated cell granulation at or near the time of metamorphosis. Experimental evidence is here an even greater necessity, and we have devoted some attention to this in recent years, as have other workers.

The situation in the larva is of particular interest in this connexion, since it provides the earliest known stage of the co-existence of thyroidal biosynthesis and a true pituitary gland. The thyroid of the adult, as is well known, develops from part of the endostyle of the larva, which is composed of glandular tracts associated with a simple epithelium (Pl. 1, fig. 1). It is now well-established that organic binding of iodine occurs in the latter, but not in the tracts, and that this leads to the formation of thyroxine and, probably, of triiodothyronine (Leloup & Berg, 1954). We are thus led to enquire as to whether any reciprocal relationship exists between the pituitary gland and the endostyle comparable to that which exists between the pituitary and thyroid of higher vertebrates.

The first relevant experimental observations were those of Knowles (1941), who injected a thyrotrophic fraction of mammalian pituitary into ammocoetes of *Lampetra planeri* and found that after three of four injections there was vaso-dilation in the endostyle and a shrinkage in length of the cells of the glandular tracts. The latter returned to normal after further injections had been given (eleven extending over one month), and he remarked on the possibility that the histological changes might be a secondary consequence of the vascular effect.

A few years later Jones (1947) reported briefly on four ammocoetes which had been kept for eleven days in 0·033 per cent thiourea solution. He found that whereas in the untreated controls there were many vacuoles in the cells

of the glandular tracts, and that the nuclei of these cells were unevenly distributed, the goitrogen-treated animals showed no such vacuoles and their nuclei were basally placed. This particular investigation seems not to have been pursued further, and it must be said that the results as reported are the exact opposite of those noted by us and by other workers in this field. Olivereau (1956), for example, found that larvae which had been immersed in 0·1 per cent thiourea for eleven days showed vacuolation and histolysis in the glandular tracts, and more particularly in the dorsal ones, and that there was hypertrophy of the epithelium.

Now at Nottingham we have been studying for some years the effects both of thiourea and of thiouracil upon the endostyle. As regards the glandular tracts, it is important to appreciate that owing to the peculiar mode of development of these, by ingrowth of cells from a particular point on the surface, the tract cells are of different ages, the oldest being in the upper half (in transverse section) and the youngest in the lower. It is thus significant that the effects of short-term goitrogen treatment are first manifested in the older cells which after two to four weeks of thiouracil treatment were found by Dr L. L. Franchi to show marked signs of increased secretory activity. The most obvious indications of this are vacuolation, the appearance of large globules of secretion, and the distal displacement of nuclei, and these features are much more conspicuous in the dorsal tracts than in the ventral (Pl. 1, fig. 2). Longer treatment results in the complete exhaustion of the older cells of the dorsal tract, with a considerable shrinkage and a complete breakdown of their normal cell relationships ; as a result of this the tract becomes greatly reduced in size (Pl. 1, fig. 3), and after ten to twelve weeks of treatment only the younger, more ventral, cells preserve a normal arrangement. The effects of thiourea are still under examination, but it can be said that the effect upon the endostyle of immersion of a larva in 0·1 per cent thiourea solution for one week is approximately equivalent to the effect of immersion in 0·03 per cent thiouracil for four weeks, the dorsal tracts again being much more affected than the ventral ones. Immersion for $6\frac{1}{2}$ weeks in thiourea solution can lead to nearly complete loss of both dorsal and ventral tracts.

Turning now to those parts of the endostylar epithelium which are known to bind iodine, Olivereau (1956), as has already been mentioned, noted hypertrophy in parts of this, and we have certainly found evidence for this after prolonged immersion of the larvae both in thiourea and thiouracil solutions (Pl. 1, fig. 3). The significance of the changes in that part of the epithelium which overlies the glandular tracts is, however, in our opinion difficult to assess, because there is also marked shrinkage of the core substance of the endostyle, and changes in the height of the epithelial cells are to be expected as a mechanical consequence of this. Despite making careful measurements of cell heights, we have found it impossible to decide with any certainty whether or not there is a true hypertrophy of the epithelium, although we would not exclude the possibility of this. On the other hand, hypertrophy of the epithelium immediately below the ventral tracts is very conspicuous and must certainly be regarded as a significant response of the cells, since in this case there are no mechanical factors which might contribute to it.

Here, then, we have some undoubted responses of the endostyle to goitrogen treatment, and some recent workers have been inclined to ascribe these to the action of thyrotrophic hormone secreted by the pituitary gland, with the implication that the feed-back relationship between the latter and thyroidal epithelium is already established at the endostylar level of evolution. In our opinion, however, the evidence for this is far from convincing. For one thing, it is to be noted that the response of hypersecretion is evoked not only in the iodine-binding epithelium but also in the glandular tracts, which do not bind iodine. Further, although Klenner & Schipper (1954) have reported that simultaneous treatment of the animals with thyroxine and goitrogen produces some reduction of the response, as would be expected if a thyrotrophin mechanism were concerned, this has not been our experience, although the thyroxine may possibly produce some effect upon the physical nature of the secretion.

Evidently the crucial test must be the study of the effect of goitrogen treatment upon hypophysectomized animals, for if these reactions are still given in the absence of the adenohypophysis they can hardly be ascribed to the action of thyrotrophic hormone. Hypophysectomy can be carried out on ammocoetes with an electric cautery (Young, 1935), and with practice it is possible to achieve complete destruction of the gland with very little disturbance to surrounding tissues, and with satisfactory survival of the animals. L. L. Franchi in a first attack upon this, and M. Sage in more extended studies, have so far found that the endostyle of hypophysectomized animals can still react to goitrogen treatment by hypersecretion and cell breakdown in the glandular tracts and by hypertrophy of the neighbouring epithelium, so that these reactions apparently can take place in the absence of the pituitary gland (Pl. 1, fig. 4). Nevertheless, it must be borne in mind that Olivereau (1956) found that they occurred in response to injections of thyrotrophic hormone, and this has been taken as presumptive evidence for control of the endostyle by the pituitary gland (Pickford & Atz, 1957), although such a response cannot be a proof that the hormone is actually secreted in the ammocoete itself. As our own experiments are still in progress it would be premature to attempt now a full assessment of them, but we are inclined to think for the present that these reponses of the endostyle merely indicate that it is an extremely labile organ, easily affected by vascular changes and stress of other kinds, although the possibility that the iodine-binding epithelium has a capacity for a more individual and specific response is certainly not excluded. That the glandular tracts are particularly labile is suggested by Knowles's (1941) observation that thyrotrophic hormone brought about vascular changes in the endostyle with shrinkage of the tract cells, but that the latter returned to normal after further injections. Equally suggestive is the fact, noted by us, that starvation of animals in clear water brings about some degree of shrinkage and breaking-up of these cells.

For the present, then, we can only conclude that we have almost everything to learn about the early evolution of the pituitary-thyroid relationship in the vertebrates, and we can only hope that the attention now being given to this problem in the cyclostomes will result in some clarification of the situation. With this in mind we may now turn to examine the teleost fish, in which group

we find a higher level of evolution and a situation which, while in some respects more clear-cut, also presents some unresolved problems.

TELEOSTEI

That thyrotroph cells exist in the pituitary gland of teleosts may be regarded as well-established. They have been identified in *Astyanax mexicanus* by Atz (1953) and in *Phoxinus phoxinus* by Barrington & Matty (1955). In the latter animal they were shown to become degranulated in precise correlation with the hypertrophy of the thyroid which occurs on treatment with goitrogens, and it seems reasonably certain that the granules of the thyrotrophs, which are readily stained with Gomori's chrome-alum-haematoxylin and with aldehyde fuchsin, represent stored thyrotrophin. The same appears to be true also of similar cells in the roach (Matty & Matty, 1959). There is here, therefore, an unquestionable pituitary-thyroid relationship, and it has been possible to demonstrate this also by means other than the use of goitrogens.

We showed some years ago (Barrington & Matty, 1954) that the activity of the thyroid of the minnow, as measured by cell-height, was increased at higher temperatures but diminished at lower ones. At that time we did not consider the means by which this reaction might be mediated, although had we done so we might have envisaged more than one possibility. One might have conceived that this was a direct response of the gland to temperature level, higher temperature in a poikilothermous animal directly increasing the secretory activity of the thyroid and lower ones diminishing it. Or, if one assumes that in some way the thyroid is implicated in cell metabolism in these fish, one might envisage that increased temperature, by stimulating metabolism, would create an increased demand for thyroid hormone and that the thyroid gland would then respond directly to this by increasing its output.

It seems clear, however, that neither of these possibilities could be a complete explanation, for Fortune has shown in a recent study (1958) that at temperatures below 10°C. the thyrotrophs of the minnow remain full and their cell contents stain readily with aldehyde fuchsin. After a fortnight at 25°C., however, there is a reduction in their granular content and in the intensity of their staining, and this is associated with a slight increase in thryoid activity, as measured on a histological basis. She therefore concludes that in this fish temperature changes affect the thyroid-pituitary relationship, and in a manner presumably different from that envisaged by Swift (1959) for the trout.

Now it is not clear in this particular instance whether the thyrotroph cells are responding directly to temperature changes, or whether their response is mediated by temperature receptors and central connexions, but it is certain that a major advantage which vertebrates gain by the possession of such trophic cells is that these can be functionally connected with the central nervous system and, through this, with the external environment. The analysis of such control systems is evidently an essential step for our understanding of endocrine cycles, as Wells emphasises in this symposium in quite a different context, and I wish, therefore, to consider whether we are yet in a position to identify in fish some signs of the existence of routes by which central control of the pituitary might be exercised. Let me add that I do so with much

diffidence, for I am able to do little more than put forward some observations without having any very satisfactory explanation for them. My excuse must be that fish have so far been a very neglected group in this context, and I hope that what I have to say may stimulate others to look for similar features ; only in this way shall we advance our knowledge in a field which is fundamental for our understanding of endocrine cycles.

First, then, let me comment briefly on the vascular relationships of the pituitary of the minnow. In higher vertebrates much has been written in recent years of the median eminence, that specially vascularized region of the infundibulum from which a system of portal vessels takes blood to the adenohypophysis. It has been vigorously argued that this portal blood carries chemical signals to the adenohypophysial cells and that these are the mediators of the nervous control to which those cells are subjected (Harris, 1955). These chemical signals seem to be conceived as rising perhaps by the discharge into the median eminence of neurosecretion directed there from hypothalamic centres, or perhaps by secretion from the glandular zone of the eminence itself. Green (1951), in his well-known survey of these relationships throughout the vertebrate series, found no trace of the eminence in teleosts, but he drew attention to the close and very characteristic interdigitation of the neurohypophysis with the adenohypophysis, and suggested that the blood vessels which extend throughout the former, and which enter into close relations with the adenohypophysial cells, were to be regarded as its equivalent. If this is so, however, one must enquire whether these vessels traverse any region which might be regarded as a possible source of chemical transmitter substances, and it was the posing of this question which directed my attention some years ago, and has continued to do so at intervals since that time, to a peculiar arrangement of vessels in the floor of the infundibulum of the minnow.

The main path for the supply of blood to the pituitary of this animal is a median ventral vessel which arises from the internal carotids at the level of the optic chiasma and runs back in the pial membrane to the pituitary stalk. On reaching this it divides to form a ring vessel which embraces the base of the stalk and which gives off numerous branches into the neurohypophysis, where they divide further and extend throughout the gland. In addition to these, however, the ring vessel also gives off delicate branches which extend upwards to form a capillary bed arching over the pituitary stalk (Barrington, 1960).

Looked at in morphological terms, this situation seems very similar in principle to Wingstrand's (1956) description of a region associated with the pituitary of the African lungfish, *Protopterus*, and which he interprets as a rudimentary eminence. He reports that the capillaries of this region are associated with processes of ependymal cells which contain a few granules of neurosecretory substance, and it is thus of interest that in the minnow there are to be found in the same general region as the capillary bed many cells which contain granules staining with chrome-alum-haematoxylin.

This situation will be described in more detail elsewhere, but unfortunately it seems unlikely that it will be possible to make any useful suggestion as to its functional significance. There is great variation in the number of the granule

cells, in the size and abundance of their inclusions, and in the degree of vascularization both in experimental and in normal animals, and further study will be necessary before any pattern which may underlie these variations is brought to light. That the situation is not peculiar to the minnow is, however, suggested by Bretschneider & de Wit's (1947) description of the blood supply of the pituitary of the teleost *Rhodeus amarus*, in which they refer to " a kind of vascular network " lying on the path of one of the infundibular arteries immediately anterior to the pituitary stalk. They were unable to suggest its significance, but concluded that it bore some relation to the hypophysis, and it certainly seems to be closely comparable with the capillary bed described here.

Before concluding, I should like to refer to one other aspect of hypophysial relationships. There have appeared in recent years several references to the possibility of the involvement of posterior lobe hormones, or material associated with them, in the regulation of adenohypophysial activity. Quite recently, for example, Martini, Mira *et al.* (1959) have reported that intravenous injection into rabbits of posterior pituitary preparations induces a significant rise in the gonadotrophic content of the urine, and they suggest that the posterior pituitary polypeptides may play some part in the physiological regulation of gonadotrophin release, while Martini, de Poli *et al.* (1959) have given evidence that vasopressin may be the hypothalamic neurohumour involved in ACTH secretion. As regards fish, Pickford & Atz (1957) have drawn attention to some little-known work of Bacon, who found that injections of mammalian posterior pituitary extracts sometimes caused maturation of prespawning animals. Wilhelmi *et al.* (1955) have reported that the muscular spasms characteristic of the spawning reflex of *Fundulus* can be elicited in that animal by injections of oxytocin and vasopressin, while Rasquin & Stoll (1955) found that pitressin injections caused an almost complete loss of glycoprotein content from the basophilic cells of the median zone of *Astyanax mexicanus*, and they regarded this as indicative of ACTH release.

These posterior lobe hormones arise in hypothalamic centres and are passed into the pituitary along nerve fibres as, or in association with, neurosecretory material which is intensely stained by chrome-alum-haematoxylin, and it is of particular interest that van Oordt (this symposium) has already discussed the possible influence of one of these centres, the nucleus preopticus, upon gonadotrophin production in amphibians. It thus seems worth while to emphasise some features of the distribution of these nerve fibres in teleosts, for they are readily seen, with their contained neurosecretion, running down the stalk and into the neurohypophysis. The nucleus lateralis tuberis is absent from the minnow, but the nucleus preopticus is well-developed, and it is doubtless from this that these fibres are derived, although we have not actually traced out their origin. The neurosecretion accumulates in the posterior region of the pituitary, to which many of the fibres are clearly directed, but it is easily demonstrated that fibres also diverge to run through the anterior and median zones of the neurohypophysis (Pl. 1, fig. 5), and that droplets of the material are also found in these parts, particularly under certain experimental conditions to which reference will be made below. Such fibres certainly run very close

indeed to the adenohypophysial cells, and da Lage (1955) has described such fibres in *Hippocampus* as actually giving rise to nerve endings applied to the secretory cells. Whether this is so also in the minnow I am unable to say, but it must be said that many of the fibres in question can certainly be seen to run right through these anterior regions and pass on to end in the posterior zone. However, it seems clear that the neurosecretory material from some of them can be deposited in neurohypophysial tissue in places where it is in almost direct contact with the adenohypophysial cells. Wingstrand (1959), in drawing attention to this morphological relationship in fish, has suggested the possibility that the neurohypophysis may here be functioning mainly as a regulator of the adenohypophysis, the more so in that the peripheral function of the neurohypophysial hormones in this group is decidedly obscure. In this context some observations made by us as a result of some minnow experiments seem of relevance. We had occasion to maintain small groups of these fish in well-aerated tanks of standing water, some of the tanks being kept in complete darkness and others being subjected to prolonged and eventually almost continuous artificial illumination. Some fish were killed after two months of this treatment and others after three months. We were looking, of course, for contrasting results from the effect of the light and dark treatment, but found, in twelve fish selected at random, that the most immediately obvious consequence was the presence after both types of treatment of extraordinarily large accumulations of neurosecretory material (Pl. 1, fig. 6), both in the stalk and extending into the anterior and median zones of the neurohypophysis, in addition, of course, to the customary conspicuous concentration in the posterior zone. Looking for other effects, it became apparent that the cyanophils of the anterior and median zones had been affected. The supposed gonadotrophs were in some fish quite well developed but showed very large vacuoles, as though they had come under some strong stimulation which had promoted the discharge of their secretion. The thyrotrophs were in some fish considerably degranulated and weakly stained, and in some were distinguishable only by the characteristic form of their nuclei, their granular contents having completely disappeared. This material awaits further analysis, and it may well be that there are other effects to be observed, but these were clear-cut and concerned cell types which are easily recognizable by well-defined characteristics.

The explanation of the results is far from obvious. Movement of neurosecretory material into the stalk in the minnow, with consequent congestion of the hypothalamico-hypophyseal region, has been reported by Arvy, Fontaine & Gabe (1954) as a consequence of immersion in saline solutions, but no such stimulus was involved in these experiments. Increased illumination is known to promote sexual maturation in this fish, but darkness delays it (Bullough, 1940a, 1940b), and it is quite unexpected that both types of treatment should result in the appearance of the gonadotrophs having been stimulated. Bearing in mind, however, that minnows have well-defined light preferences (Woodhead, 1956), it seems possible that in this experiment both continuous darkness and prolonged illumination were stressful conditions, in which case one could speculate that under such circumstances the hypothalamus would be stimulated, with the consequent release of neurosecretion which might

then activate some of the adenohypophysial cells with the discharge of their secretion. I must emphasise, however, that there is at present no evidence that such a causal chain actually operates in the minnow, and the point is raised here because it is an example of a possible hypophysial relationship which, especially in the light of the other recent work cited, ought to be rigorously tested, and indeed we have the problem in hand at this moment. Wingstrand (1959) has pointed out that if transmitter substances are released from the neurosecretory fibres, they might reach the secretory cells either by direct contact or by humoral transmission in the capillary plexus, and in this connexion it is worth noting certain aspects of the distribution of the cell types in the adenohypophysis which can easily be lost sight of if too much weight is placed upon its regional differentiation. To my mind, for example, the significant feature of the distribution of the thyrotroph cells of the minnow is not their restriction to any particular zone or zones, but the fact that most of them are situated at or close to the border between the adenohypophysis and the major digitations of the neurohypophysis ; it is difficult to avoid the suspicion that such an arrangement must be indicative of some physiological relation between those cells and some component of the neurohypophysis, either vascular or neurosecretory. Indeed, it is likely that such relationships may be determining factors in the development of the pattern of cell differentiation in the pituitary and it is certain that if we knew more of the operation of such factors during early stages of development we should be far advanced in our understanding of the mode of functioning of the gland.

In conclusion, may I say that I am well aware that I have provided in these comments a very great deal of speculation, but I believe that such is one of the proper functions of contributions to symposia such as this. I shall be well satisfied if I have succeeded in persuading some other workers to search over a wider range of species in the lower vertebrates for clues to the elucidation of these fascinating problems of the regulation of endocrine cycles.

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EXPLANATION OF THE PLATE

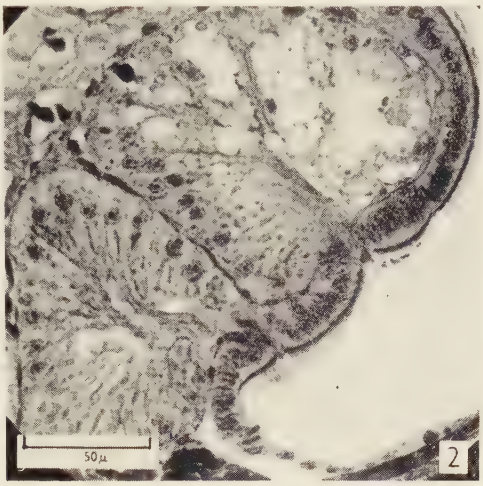
Fig. 1.—Transverse section of the left half of the anterior end of the endostyle of an ammocoete larva, showing the paired dorsal and ventral glandular tracts and the simple epithelium extending from them. (This, and the succeeding three figures, are from an investigation by L. L. Franchi).

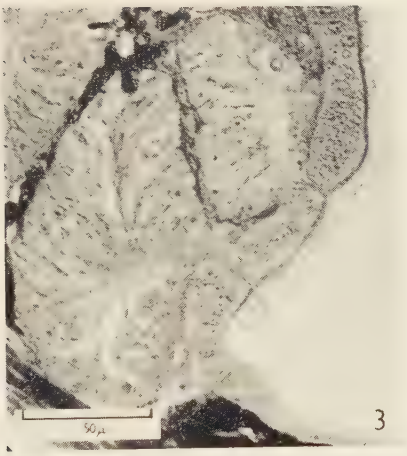
Fig. 2.—Dorsal and ventral tracts, corresponding to those on the right side in fig. 1, of an ammocoete which had been maintained for four weeks in a thiouracil solution. Note the exhaustion of the more dorsal (older) cells of the dorsal tract.

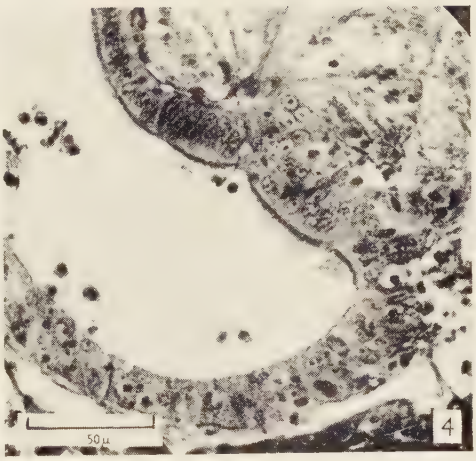
Fig. 3.—Dorsal and ventral tracts of an ammocoete which had been maintained for ten weeks in a thiouracil solution. Note the marked shrinkage of the dorsal tract. The simple epithelium appears to be hypertrophied, particularly towards the upper and lower edges of the field.

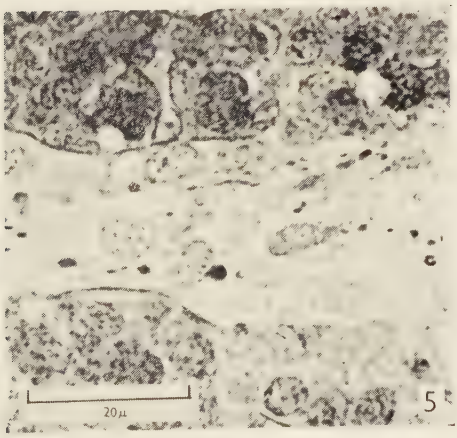
Fig. 4.—Part of the dorsal and ventral tracts and adjacent epithelium, corresponding to the left side of fig. 1, of an ammocoete which had been maintained in thiouracil solution after total hypophysectomy. Note the very marked hypertrophy of the epithelium and the degeneration of the tracts.

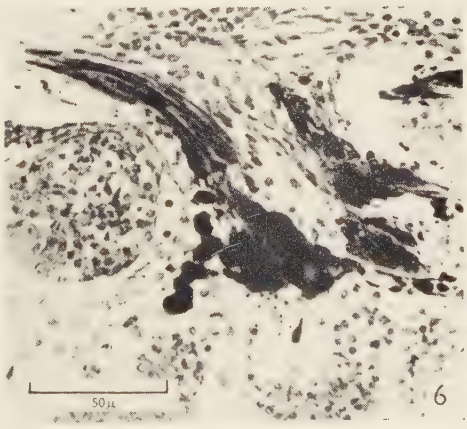
Fig. 5.—Part of the anterior region of the pituitary gland of the minnow (chrome-alum-haematoxylin and phloxin). The transverse light band is neurohypophysial tissue, and it contains darkly staining drops and streaks of neurosecretory material, either separate or in nerve fibres ; these are sometimes close to the adenohypophysial cells, as in the upper right corner of the field. (This and the suceeding figure are from an investigation carried out with Miss J. M. Plumtree).











Some problems of adenohypophysial relationships in cyclostomes and fish.

Fig. 6.—The stalk and adjacent region of the pituitary gland of a minnow (chrome-alum-haematoxylin and phloxin). Darkly staining neurosecretory fibres, presumed to be derived from the nucleus preopticus, are entering the stalk from the left, and large accumulations of neurosecretory material lie in the neurohypophysial tissue beneath them. Adenohypophysial cells lie below these and to the left of them, and show marked signs of vacuolation. The animals from which this and the preceding section were taken had been maintained in darkness for 68 days ; for further explanation see text.

OPTIC GLANDS AND THE OVARY OF *OCTOPUS*

BY

M. J. WELLS

Department of Zoology, Cambridge

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INTRODUCTION

The onset of sexual maturity in *Octopus* is determined by a secretion released into the bloodstream by the optic glands ; production of this secretion is regulated by an inhibitory nerve supply originating in the posterior part of the supraoesophageal mass of the central nervous system (Wells & Wells 1959).

In the present account evidence for this statement is reviewed and additional experiments are reported showing that in young octopuses the optic glands become capable of secreting before the ovary is competent to respond ; the oviducts, ovisac and oviducal glands of these animals ripen even when the ovary itself does not and this suggests that their condition is directly dependent upon optic gland secretion rather than upon any product of the ripening ovary.

All the experiments to be described were made with *female* octopuses, because the effect on the ovary of changes induced in the optic glands are particularly rapid and obvious. But male octopuses also have optic glands and since the state of the testis is clearly related to the condition of these (Wells & Wells 1959) there is no reason for supposing that glandular control of the onset of sexual maturity is in any way different in the two sexes (but see p. 102). The system to be described seems likely to prove general for all metacephalopods (that is, all living cephalopods except *Nautilus*), since all those so far examined have optic glands.

MATERIAL AND METHODS

All the experiments summarised here were made at the Stazione Zoologica di Napoli with octopuses collected in and around the Bay of Naples, the animals being caught, kept in individual aquaria and fed upon pieces of freshly killed fish or Crustacea, as described by Boycott (1954). Nearly all of the animals were used in training experiments, most of which have already been described elsewhere (see Wells 1959 for references), and all but the control animals were subjected to brain operations in which parts of the centra lnervous system were removed and/or the optic nerves severed under urethane anaesthesia ; details of operational techniques are given in Wells & Wells (1956, 1957).

The length of time that octopuses were kept after operation depended upon the nature of the training experiments made, the majority of the animals being killed at the end of experiments lasting from two to six weeks so that their brain lesions could be checked from serial sections ; a few were kept for relatively long periods after the end of training and some of these (animals of 700-900 gr.) laid eggs. In all cases the animals, most of which grew considerably during their periods of captivity, were weighed when killed, intact body weight and the weight of the excised ovary (with its ducts) being recorded in each instance.

The octopuses kept in aquaria for use in training experiments were from two size ranges, 15-60 gr. (data from twenty-nine animals) and 200-1000 gr. (data from 177 animals) ; in addition data were collected from a few larger animals fresh from the sea. In Tables 1-3 and in the figures most individual octopuses are distinguished by the serial number that they carried in the original protocols of training experiments made with them. Each such number is prefixed by A, B, C . . . F showing the year 1954-9 in which the animal was killed. Other animals, not used in training experiments, are identified by a letter reference or more simply by their weight, in each case with the usual prefix giving the year in which the record was made.

The standard histological techniques used to examine brains, optic glands and gonads are listed in the text.

ANATOMY OF THE PARTS CONCERNED

(a) *The brain and the optic glands*

The central nervous system of *Octopus* is concentrated into a compact mass around the oesophagus (Fig. 1). This brain can be subdivided into about thirty anatomically distinct regions and it is possible to ascribe functions to most of these (for a further description of the anatomy of the brain, and of the evidence for the separate functions of its parts, see Boycott & Young 1961). For present purposes it suffices to distinguish an upper, supraoesophageal part of the brain, to which the optic lobes are attached by stalks on either side, and a suboesophageal region. The supraosophageal lobes include sensory integrative and higher motor control areas, and with two exceptions do not give rise to peripheral nerves, but act through the lower motor centres in the suboesophageal part of the brain. The two exceptions are the buccal lobe at the anterior extremity, which gives rise to motor nerves to the buccal mass, and the subpedunculate lobe, at the posterior extremity of the supraoesophageal

part of the brain. This lobe (Fig. 1) is a small bilateral structure, having its own neuropil of fine fibres surrounded by small nerve cell bodies (nuclei $<5\mu$ in an animal of 500 gr., with very little cytoplasm) ; it is remarkable in giving rise to two distinct efferent nerves, running into the orbit on each side of the brain. The subpedunculate nerve, from which the lobe derives its name, does not concern us here. It is a curious structure, running out along the floor of the orbit; Boycott & Young (1956) have described it and tentatively suggest

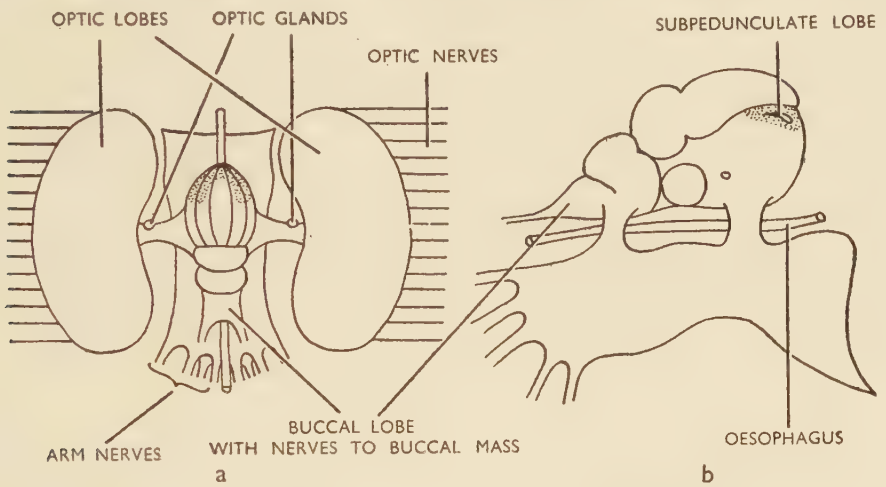

Fig. 1.—Anatomy of the brain of *Octopus*. (a) As it would be seen from above after removal of the cartilage surrounding the central mass, and (b) in vertical longitudinal section. In an animal of 500 gr. the supraoesophageal part of the brain would be about 4 mm. long. The subpedunculate lobe is shaded in both diagrams.

that it serves in some way to regulate pressure in the anterior chamber of the orbit. The other nerve arising from the subpedunculate lobe runs to the optic gland (Boycott & Young 1956, Wells & Wells 1959). There is no evidence that either of these nerves form part of a neuro-secretory system ; sections through the subpedunculate lobe, stained with Gomori chrome-haematoxylin do not appear to include cell bodies or axons containing neurosecretory material.

The optic glands are small, spherical bodies, yellowish in colour in immature octopuses, lying on either side of the brain on the optic stalks close to the optic lobes (Fig. 1). Each consists of a mass of stellate glandular cells enmeshed in a network of connective tissue ; the gland cells are not arranged in vesicles but form an irregular network with spaces between, through which an abundant blood supply runs from a branch of the cephalic artery arriving along the optic stalk.

The gland is structurally uniform throughout and only two types of cells are present. One of the two, with small regularly-shaped ovoid nuclei and scanty fibrous cytoplasm (the " supporting cells " in Figs. 3 and 5) does not alter in size or staining properties when the gland enlarges, and concerns us here only inasmuch as its appearance can be used as a control for the staining properties of the " chief cells " (the second type of cell) which *do* alter when the gland enlarges after various experimental treatments (Figs. 3 and 5).

The chief cells are readily distinguished from the supporting cells by their much greater size, large ovoid nuclei with prominent nucleoli, and irregular cytoplasm, staining mauve with Azan in contrast to the clear blue of the supporting cells. Nuclei, nuclear granules and nucleoli stain dark blue with Azan in the chief cells, again in contrast to the supporting cells which have no obvious nucleoli and larger red-staining granules. Staining the chief cells with Schiff's reagent and Methyl green-Pyronin (before and after ribonuclease digestion) indicates that DNA is limited to the nucleoli and shows that RNA is present in high concentration in both nuclei and cytoplasm ; in the supporting cells Methyl green-Pyronin stains only the nuclei and the reaction is not affected by ribonuclease digestion. Since high RNA-content is characteristically associated with protein synthesis (Brachet 1957) it seems likely that the chief cells secrete protein and that the supporting cells do not. The latter are probably simply connective tissue elements providing a framework in which the chief cells are lodged.

Following various experimental treatments to be described, and in animals maturing normally at a weight of 2000 +gr., the optic glands are found to contain vesicles of a yellowish secretion, at first appearing in the cytoplasm of the chief cells and later in the spaces between them. It will be shown that this product is immediately responsible for the condition of the ovary and of its ducts.

(b) *The ovary and its ducts.*

Octopuses have a single ovary and two oviducts. The ovary lies in a sac at the extreme hind end of the body, where it can be seen when the mantle cavity is opened ; the oviducts run forwards from this and open on either side of the septal muscle (Fig. 2).

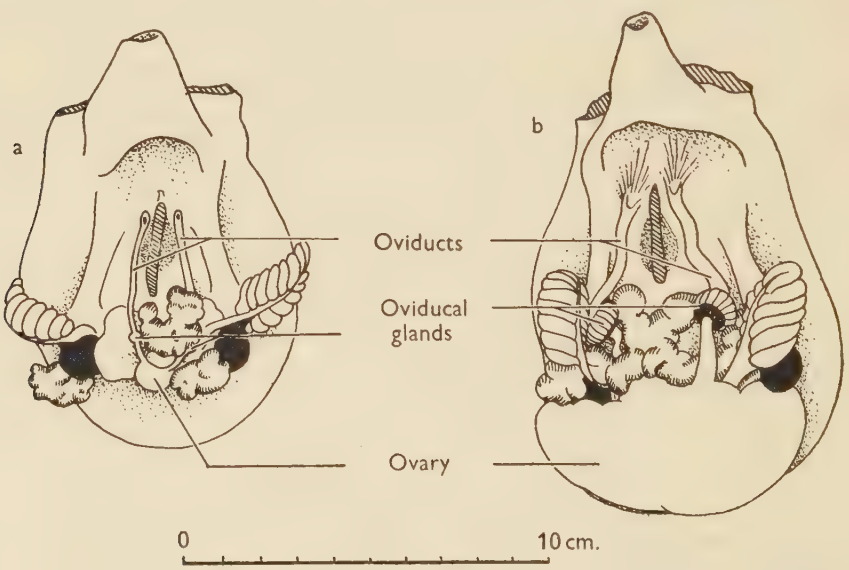

Fig. 2.—Condition of the ovary and its ducts in normal and operated animals. The head and arms have been cut off, the mantle folded back after severing the septal muscle, and some of the kidney dissected away. (a) A control animal of about 500 gr. and (b) an octopus of similar size thirty-seven days after an operation in which a central brain lesion including the subpedunculate lobe was made, causing precocious maturation of the ovary (from Wells & Wells 1959, *J. exp. Biol.* **36** : p. 5).

In immature octopuses the ovary and its ducts are always very small, together constituting about 1/500 th of the total body weight of the intact animal. As the animal approaches maturity, whether in the normal way at a body weight of 2000 gr. or more, or precociously as a result of various experimental treatments to be described, the ovary increases enormously is size, and eventually fills much of the hinder part of the mantle cavity (Fig. 2b) ; it may then constitute as much as 1/5 th of the total body weight.

As the ovary ripens, so do its ducts. The oviducts become swollen, and their external apertures fan out ; the oviducal glands increase greatly in size and change colour from a uniform white to pale yellow and brown (Fig. 2b). The structure of these and of the ovary is further described below, together with details of some of the histological changes that accompany maturation.

A SUMMARY OF THE EVIDENCE FOR HORMONAL CONTROL OF SEXUAL MATURITY BY THE OPTIC GLANDS

(a) *Evidence from the condition of the optic glands in mature and immature animals.*

In octopuses of less than 1000 gr. the optic glands are always small and pale yellow in colour, whatever the time of year. When sectioned and stained it is clear that these glands are inactive, or at least not producing secretion faster than it can be carried away in the bloodstream.

Fig. 3.—Condition of the optic glands in unoperated animals of (a) 37 gr. (octopus EKL), (b) 610 gr. (C610) and (c) 3320 gr. (F3320) ; (d) shows cells from an optic gland of an octopus of 570 gr. (D16) blinded by optic nerves section fifty-two days previously. In (c) and (d), animals with ripening ovaries (table 1), vesicles of secretion are found in the chief cells of the optic glands. s=supporting cell nucleus (connective tissue element), c=chief cell nucleus and v=vesicles of secretory product of the chief cells. (a), (b), (c) and (d) are all drawn to the same scale.

Optic glands are present in octopuses weighing as little as 13 gr., the smallest animal examined. In these small animals the histological appearance of the gland is in every way similar to that of glands in larger immature octopuses from the 200-1000 gr. range (Fig. 3). The ratio of optic gland volume to body weight is at first relatively great (as is brain/body weight) but decreases progressively as the animal grows until a weight of 2000 gr. or more is reached, when the relative size of the gland again increases (Table 1).

TABLE 1

Optic gland and ovary size in octopuses maturing normally and as a result of experimental treatments.

Animal	Days since operation	Body wt. (gr.)	Ovary wt. (gr.)	Gonad/body wt.	Vol. of optic gland	Optic gland vol./body wt. x 1000
CONTROLS						
EKL	—	37	0·051	0·0014	0·066	1·79
C610	—	610	1·0	0·0016	0·41	0·67
C178	—	630	1·0	0·0016	0·44	0·70
B141	—	1140	2·7	0·0024	0·44	0·39
F3320	—	3320	150·0	0·0465	6·63	2·00
ANIMALS BLINDED BY OPTIC NERVES SECTION						
C197*	15	460	see below		0·21	0·46
C144*	31	490			0·27	0·55
D16	52	570	37·5	0·0659	0·93	1·64
ANIMALS WITH CENTRAL LESIONS INCLUDING THE SUBPEDUNCULATE LOBE†						
A137	5	460	0·9	0·0020	0·84	1·84
C197*	15	460	3·5	0·0076	0·70	1·52
C144*	31	490	84·3	0·1730	2·30	4·70
C16	45	650	74·0	0·1140	4·24	6·50
C27	64‡	890	117·7	0·1323	10·00	11·24
EK1C	45	52	0·530	0·0100	0·840	16·20

* C144 and C197 had unilateral central lesions and appear twice in this table, one optic gland in each case having enlarged as a result of a central lesion, the other more slowly as a consequence of blinding ; it has been shown elsewhere that there is no cumulative effect of the two operations upon the rate of ovarian enlargement (Wells & Wells 1959).

† All of these animals, except EK1C, also had the optic nerves cut.

‡ C27 was subjected to two successive operations. At the first (sixty-four days before the animal finally laid eggs) the optic nerves were cut ; at the second (thirty-nine days later) a central lesion was made ; the ovary weight recorded here does not include the weight of the eggs that were laid.

Octopuses maturing normally at 2000+gr. with ripening ovaries have, therefore, relatively large optic glands. On sectioning it can be seen that the increase in glandular volume is due to an increase in the size of the chief cells (Fig. 3) ; no mitotic figures can be found and it seems probable that the observed enlargement of the gland is due solely to growth by individual cells. Within the chief cells of ripening octopuses there are commonly vesicles of a yellowish substance (Fig. 3) not present in the glands of immature specimens. Enlarged glands from ripe animals stain at least as darkly with Methyl green-Pyronin as the glands of immature specimens, and since the chief cells are much larger this must indicate a considerable increase in the total RNA present in the gland.

(b) *Evidence from experiments in which precocious enlargement of the ovary and optic glands was induced by surgical treatments* (from Wells & Wells 1959)

Precocious maturation of the gonad can be caused by blinding octopuses (optic nerves section or section of the optic tracts peripheral to the optic glands) or by removal of the subpedunculate lobe from the supraoesophageal part of the brain. It can also be caused by section of the optic stalks central to the optic glands (provided the optic lobes and glands are left in) or by section of the subpedunculate-optic gland nerve anywhere along its length.

Fig. 4 summarises the results of sixty-five experiments in which precocious enlargement of the ovary was induced, either by blinding or by a central lesion including the subpedunculate lobe, in animals weighing between 250 and 1000 gr. that would normally be immature. It can be seen that enlargement of the ovary following these treatments was in many cases very rapid and very considerable, the weight of the ovary rising from approximately 1/500th to 1/5th of the total body weight within four to five weeks of operation. Details of these experiments and of the lesions made have been given in Wells & Wells (1959).

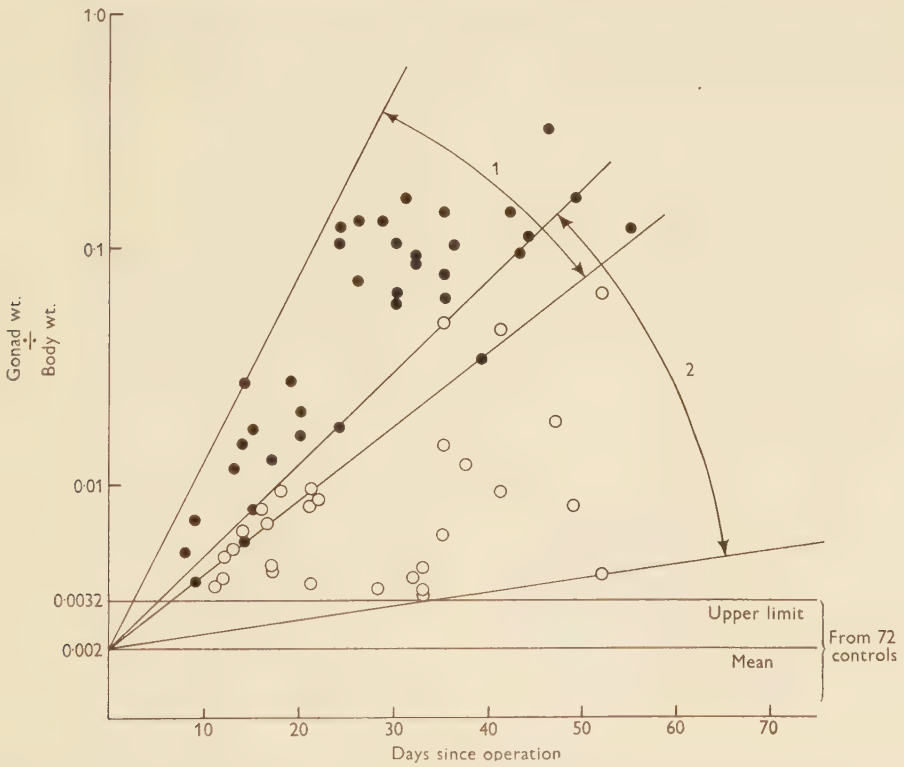

Fig. 4.—A summary of the results of sixty-five experiments in which precocious enlargement of the ovary was induced (1) by central brain lesions including the subpedunculate lobe (indicated ●) and (2) by section of the optic nerves (indicated O). It can be seen that very rapid enlargement of the ovary follows these experimental treatments, the ovary increasing from about 1/500 th to as much as 1/5 th of the total body weight within six weeks of operation. In every one of these experiments one or both of the optic glands was also found to be enlarged (from Wells & Wells 1959. *J. exp. Biol.* vol. 36, p. 15).

In every one of the experiments in which the ovary was enlarged one or both of the optic glands was enlarged as well. In no instance were the glands enlarged in control animals. Subsequent sectioning of the brains of the experimental animals showed that unilateral enlargement of one optic gland only occurred when the operation was incomplete on one side ; optic glands did not enlarge on sides where any optic nerves remained or where the central lesion failed to include the whole of the subpedunculate lobe.

No difference in the rate of enlargement of the ovary was found in animals showing unilateral and bilateral enlargement of the optic glands as a result of central lesions. If, as seems probable from the experimental evidence, a secretion of the optic glands is responsible for the enlargement of the ovary, one would expect to find this secretion produced in excess in the optic glands of operated animals. This expectation is confirmed histologically. In animals with central lesions interrupting what must be an inhibitory nerve supply to the optic glands, the cytoplasm of the chief cells and the spaces between the cells speedily (five to six weeks) become choked with globules of secretion, which is evidently produced faster than it can be carried away in the bloodstream (Fig. 5b,c). There is no such excess of secretion in the glands of animals ripening normally or in octopuses maturing precociously (though more slowly than after central lesions) as a result of peripheral blinding (Fig. 3c,d) ; in both

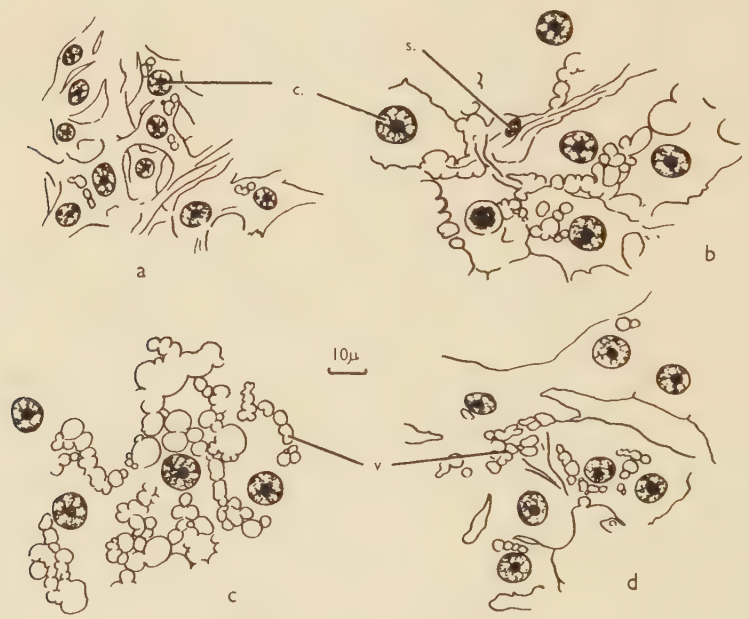

Fig. 5.—Condition of the optic glands in animals with central brain lesions at various periods after operation. (a) At five days (animal A137 of 460 gr.), (b) at thirty-one days (C144, 490 gr.) and (c) at sixty-four days (C27, 890 gr.—this animal laid eggs). (a), (b) and (c) show progressive enlargement of the chief cells and accumulation of their secretory product. (d) shows a part of an optic gland from a very small animal, EK1C (52 gr.) forty-five days after operation, and may be compared with Fig. 3a, drawn from a control animal of approximately the same size. s., c., and v. as in Fig. 3.

these the enlarged optic glands secrete little or no faster than their product can be carried away in the bloodstream, and remain yellow in colour, in contrast to the bright orange of the glands of animals with central lesions.

When central lesions including the subpedunculate lobe are made and the animals killed within seven days of operation, enlargement of the ovary is not obvious on dissection, but the optic glands are already recognizably larger than those of controls (measurements from one such animal, A137, are included in table 1). On sectioning it is found that such glands already contain droplets of secretory material (Fig. 5a). There is therefore evidence from timing that optic gland secretion is causally linked with the condition of the ovary which does not begin to enlarge until after secretion by the optic glands has begun.

(c) *Evidence from the effects of removal of the optic gland* (from Wells & Wells 1959).

Mature female octopuses with ripening ovaries tend to be rather large and inconvenient to keep in aquaria and no experimental removals of optic glands from normally maturing animals have yet been made.

Experiments have, however, been made with immature animals. Removal of the optic glands from such octopuses has no obvious immediate effect upon the animals, which continue to feed and grow normally (Sereni 1930, Callan unpublished, quoted in Boycott & Young 1956). Nor is there any very obvious effect upon the size of the ovary ; three otherwise unoperated animals had ovary weights slightly above the mean for controls when killed three to six weeks after optic gland removal and a fourth animal, killed after six weeks, an ovary weighing somewhat less (Table 2).

TABLE 2

The effect of removal of the optic glands upon ovary size.

Animal	Days since operation	gonad/body wt. ratio	ratio above or below mean (0·0020) for 72 controls
UNBLINDED ANIMALS WITHOUT CENTRAL LESIONS			
A183	17	0·0024	above
C62	45	0·0022	above
C67	45	0·0032	above
A193	47	0·0014	below
ANIMALS WITH THE OPTIC LOBES REMOVED			
C44	48	0·0021	above
C56	50	0·0018	below
ANIMALS WITH CENTRAL LESIONS INCLUDING THE SUBPEDUNCULATE LOBE			
B173	10	0·0022	above
C85	14	0·0027	above
C116	32	0·0014	below
C119	55	0·0011	below
C121	55	0·0014	below

The ovary of C121 was sectioned and found to contain developing ova of normal size (Table 3).

Optic gland removal does, however, effect the outcome of experimental procedures that would otherwise induce precocious maturation of the ovary.

If the optic glands are removed at the same time as the animal is blinded or centrally damaged, no subsequent enlargement of the ovary occurs. Thus two such animals blinded by optic tract section peripheral to the optic glands (this method of blinding has the advantage of certain completeness and normally leads to enlargement of the ovary at the same rate as peripheral optic nerves section), killed seven weeks after operation, both had small ovaries, one slightly above and the other below the mean size for controls. Five further octopuses were kept for from two to eight weeks without enlargement of the ovary after operations in which central lesions were made including the whole of the subpedunculate lobe on both sides of the brain (Table 2). The ovary from one of these animals (C121, kept for eight weeks after operation) was sectioned and found to contain ova of a size and appearance normal for controls of similar body weight (Table 3).

It appears therefore, that though the optic glands must be present for maturation of the ovary to take place they are probably not essential to the maintenance of the immature ovary which does not regress if they are removed. This is consistent with the apparent absence of secretory material in the unenlarged glands of immature octopuses.

CHANGES IN NORMAL AND PRECOCIOUS MATURATION OF THE OVARY AND ITS DUCTS

(a) *Changes in the condition of the ovary itself*

(1) Changes in normal and experimentally induced maturation of the ovary in animals weighing more than 250 gr.

The gross anatomy of the ovary of *Octopus* is illustrated in Fig. and 6a b, which are drawn from an unoperated immature animal of 610 gr. (a section through part of one of the optic glands of this octopus is included in Fig. 3). The germinal epithelium is much folded, so that the developing oocytes, which remain attached by stalks to the underlying connective tissue, hang in bunches from the anterior wall of the ovisac. In such immature octopuses the developing ova vary considerably in size, and in this instance the largest seen measured 0·112 mm. in diameter. Each ovum has a large nucleus, with one or two prominent nucleoli lying in the middle of a dense, finely granular cytoplasm ; this is surrounded by follicular cells, folds of which intrude into the developing egg as it gets larger (Isgrove 1909).

As the animal matures the eggs increase and become more uniform in size, until the mature ovary is packed with ova, all measuring approximately 3×1 mm., attached like bunches of grapes to the anterior wall of the ovary, which now bulges into the mantle cavity (Fig. 2b). When full sized the mature eggs break away from the epithelial wall, and pass down the oviducts, through the oviducal glands, where they are fertilized and the eggshells hardened, to be attached in elongate bundles to the vertical or overhanging surface selected as a nesting site. A very large number of eggs is laid over a period of seven to ten days.

The histological appearance of ovaries ripening precociously as a result of experimental treatments does not differ from that of ovaries ripening normally in larger animals. The eggs increase in size as before and are laid at full size.

Changes resulting from central lesions first become apparent about two weeks after operation by which time the largest ova are already appreciably greater than the largest in controls of similar size (Table 3). Eggs laid by precociously ripened animals may be fertile and would perhaps always be so were the animals not kept in isolation. Precociously ripened females, moreover, behave quite normally, brooding their eggs and refusing all food during the incubation period (Wells & Wells 1959).

TABLE 3

Size of ova and ovaries in animals maturing normally and as a result of experimental treatments.

Animal	Days since operation	Body wt. (gr.)	Ovary wt. (gr.)	Gonad/body wt.	Diameter of largest ovum (mm.)
CONTROLS					
(1) Immature animals with small optic glands.					
EA	—	18	0·032	0·0018	0·065
EKL	—	37	0·051	0·0013	0·077
C610	—	610	1·0	0·0016	0·112
EE	—	1120	3·6	0·0032	0·15
(2) Maturing animals with enlarged optic glands.					
E2000	—	2000	130	0·0650	" Eggs not quite full size "
F3320	—	3320	150	0·0465	
ANIMALS BLINDED BY OPTIC NERVE SECTION					
C108	13	380	1·5	0·0040	0·22
C30	48	720	5·8	0·0081	0·23
A158	79	640	9·0	0·1041	0·38
ANIMALS WITH CENTRAL LESIONS INCLUDING THE SUBPEDUNCULATE LOBE					
(1) Octopuses from the 200-1000 gr. range.					
C158	3	520	2·0	0·0039	0·17
D39	15	220	3·7	0·0168	0·47
C144	34	490	84·3	0·1730	0·90
C16	45	650	74·0	0·1140	0·90
C84	50	550	89·3	0·1635	0·84
C27	64*	890	117·7	0·1323	1·00
(2) Octopuses of < 60 gr. in which the ovary did not develop.					
EK1A	37	39	0·920†	0·236	0·015
EK1C	45	52	0·530†	0·102	0·035
(3) Octopus with the optic glands removed.					
C121	55	580	0·8	0·0014	0·080

* C27 was subjected to two successive operations ; the optic nerves were cut sixty-four days and a central lesion made twenty-five days before this animal laid eggs. The weight of the ovary of C27 does not include eggs already laid.

† These weights are not entirely reliable, because of escape of some of the liquid from the ovisac on dissection.

2. Changes following central lesions to the brains of octopuses weighing less than 60 gr.

When central lesions including the subpedunculate lobe are made in the brains of very young octopuses (animals weighing less than 60 gr. in June—July were probably spawned in the previous summer (Wells & Wells 1959)), the optic glands enlarge and begin to secrete in the normal way (Fig. 5d) but the ovary

fails to respond. In such instances the ovisac, oviducts and oviducal glands all undergo histological changes of the type normally associated with maturation of the ovary, and increase greatly in size and weight (Fig. 6c, d and Fig.7), but the germinal epithelium itself remains unaffected ; the ovary in such animals can

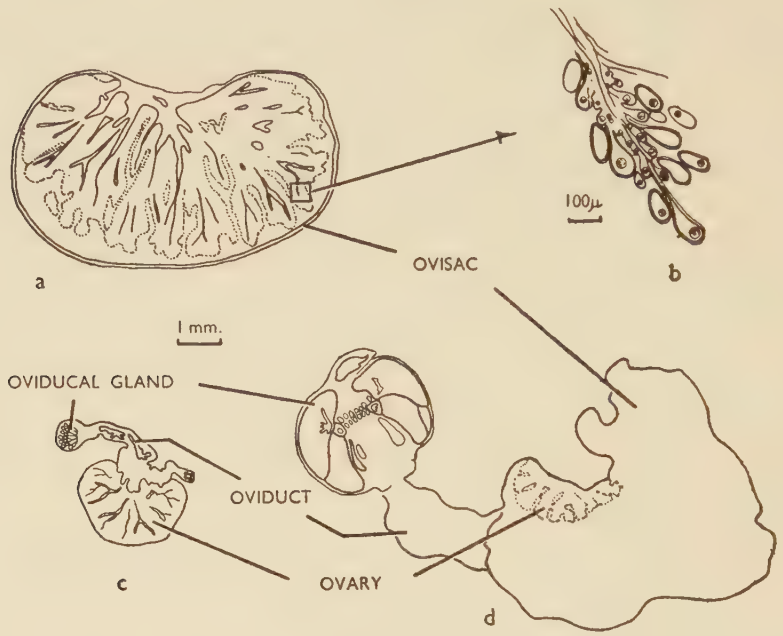

Fig. 6.—(a), (b). Structure of the ovary of an immature octopus of 610 gr. (C610) ; the germinal epithelium is much folded and the developing oocytes hang in bunches from the anterior border of the ovisac. (c) and (d), drawn to the same scale as (a) show the condition of the ovary and one oviduct in, respectively, a control animal of 37 gr. (EKL) and in an octopus of 39 gr. (EK1A) thirty-seven days after an operation in which a central lesion was made. In EK1A the ovisac, oviduct and oviducal gland are much enlarged, but the ovary is evidently not yet competent to react to the optic gland secretion.

be seen on dissection as a small pinkish mass lying in the enlarged transparent fluid-filled ovisac (Fig. 7). This mass consists mainly of connective tissue. In sections no well-formed stalked ova are seen and there is an almost total absence of the mauve-staining (Azan) yolk that characteristically surrounds each developing egg nucleus, even in very small animals. Large nuclei, of a similar size and in a similar position to those surrounded by yolk in controls can be found, but only very occasionally is there any trace of yolk around them. Quite what has happened in these instances is not clear and requires further investigation ; it would appear that the oocytes develop normally, but the follicular cells do not ; at all events it is apparent that the ovary is in some way not competent to react to optic gland secretion, and it seems reasonable to suppose that since oviducts, ovisac and oviducal glands nevertheless develop normally these are directly affected by optic gland secretion and not dependent upon a secondary hormone released by the ripening ovary. This would be in

keeping with Taki's (1944) observation that removal of the ovary is not followed by degeneration of the oviducts. Evidently the female gonad, at least, does not produce a sex-hormone in cephalopods.

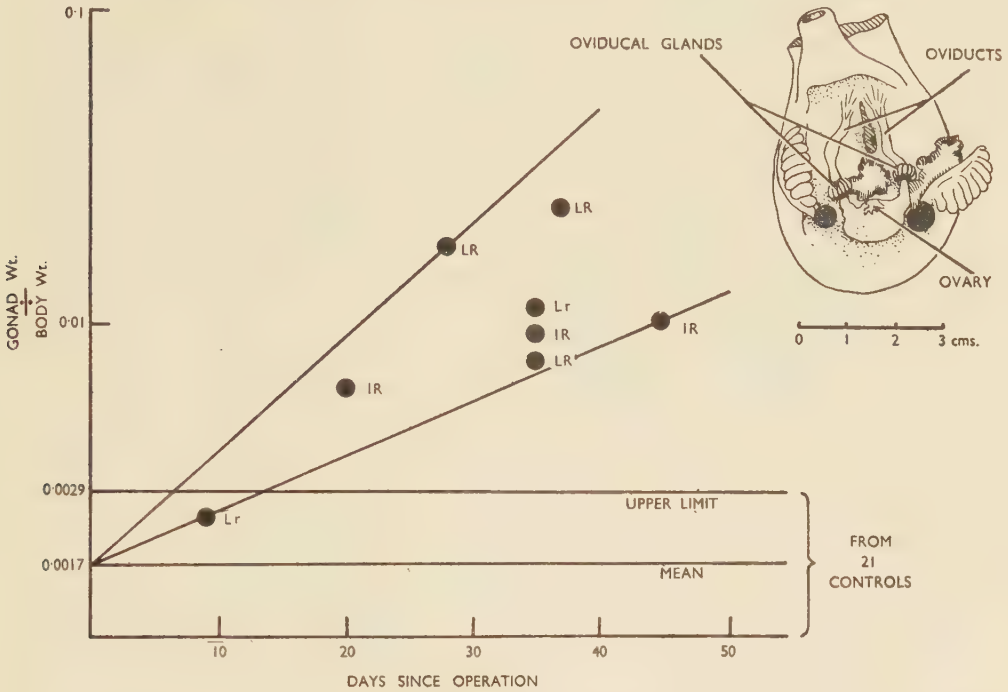

Fig. 7.—A summary of experiments in which precocious enlargement of the ovisac and oviducts was induced by surgical removal of parts from the supraoesophageal mass, including the subpedunculate lobe on one or both sides of the brain. These experiments were made with small octopuses weighing less than 60 gr. when killed ; at this size the ovary itself is evidently not competent to react to the secretion of the optic glands one or both of which was in all instances enlarged. The condition of the optic glands in each animal is recorded ; L and R respectively indicate left and right glands, enlarged, and l and r glands *not* enlarged. " Ovary weight " plotted here includes the weight of the oviducts, their glands and of the enlarged fluid-filled ovisac. Insert shows the appearance of the ovary and its ducts in animal EK1C, displayed as in Fig. 2 (from a Kodachrome transparancy).

(b) *Changes within the oviducal glands*

Ripening of the ovary is accompanied by changes in the size and appearance of its ducts. The oviducts expand—the lumen of the oviduct of a precociously ripened animal of 650 gr. was 1·4 mm. in diameter, that of a control animal of similar size 0·3 mm.—their walls become much thickened with connective tissue and hard to the touch, and their distal ends fan out (Fig. 2b).

The oviducal glands undergo even more extensive changes. In immature animals these glands are small, whitish swellings about twice the diameter of the oviduct (Fig. 2a). Internally they have a complex structure, being divided radially into a number of segments, and longitudinally into three distinct

parts, two of which are clearly glandular, the third, nearest the ovary, a sac with relatively thin walls and a large lumen ; ducts from all three divisions converge upon the oviduct. All these features are best seen in the mature gland, although they are traceable even in the oviducal glands of unoperated animals weighing less than 60 gr. (Fig. 8).

Enlargement of the optic glands is invariably accompanied by enlargement of the oviducal glands, even in very small octopuses in which the ovary itself fails to respond to the optic gland secretion. The histological changes taking place in the enlarging oviducal glands appear to be identical in octopuses throughout the size range (13-3320 gr.) studied. All parts of the oviducal glands enlarge as the gland grows bigger, and the walls of the secretory regions become very greatly folded to form a complex system of ducts (Fig. 8e); the internal radially segmented structure becomes obvious from the outside and the secretory part of the gland furthest from the ovary becomes orange or brownish in colour (in formalin-fixed material ; in life this part of the gland is white, in contrast to the other glandular region which appears darker by comparison—as in Fig. 2b). If cut in half the internal part of this section of the gland (Gl. 2 in Fig.8) is found to be pale yellow, but rapidly darkens even after long fixation, so that within a few hours it is uniformly orange or brown. On histological examination this region can be shown to secrete a strongly reducing substance, formed in the cells and released into the lumen of the gland as spherical droplets of rather constant small size (Fig. 8f). This substance stains dark red with Azocarmine and dark brown or black in ammoniacal silver nitrate ; in Millon's reagent a red colour is produced, in contrast to the rest of the oviducal gland which colours hardly at all.

Of the other glandular element in the oviducal glands (Gl. 1 in Fig. 8) only the nuclei stain red with Azan, and the clear cytoplasm does not reduce silver nitrate. In ripe specimens the cells of this gland are crammed with vacuoles of a transparent or whitish substance (Fig. 8g), and the walls of the ducts of the gland become tattered as this is released.

No attempt has so far been made to identify the products of the oviducal glands. The two-gland system does, however, appear from the present cursory examination to have so much in common with systems producing and tanning proteins elsewhere (the byssus of *Mytilus*, for example, see Brown 1952) that there is little doubt that a more thorough examination would reveal yet another protein tanning system, with the gland nearest the ovary producing the bulk of the protein of the eggshell and the more distal gland an aromatic tanning agent.

The third, sac-like, element in the oviducal glands does not change much in histological appearance (there is some slight thickening of its walls) as the gland ripens and in nearly all cases examined, including animal C27 which laid and brooded strings of infertile eggs, was empty. In a single large (3320 gr.) mature octopus the sacs were packed with thread-like structures, the ends of which, buried in the walls of the vesicles, stained deeply with Azocarmine—a nuclear stain. It seems likely that these threads were sperm, and that the vesicular elements in the oviducal glands are spermathecae. This hypothesis would be consistent with their position—opening into the lumen of the oviduct

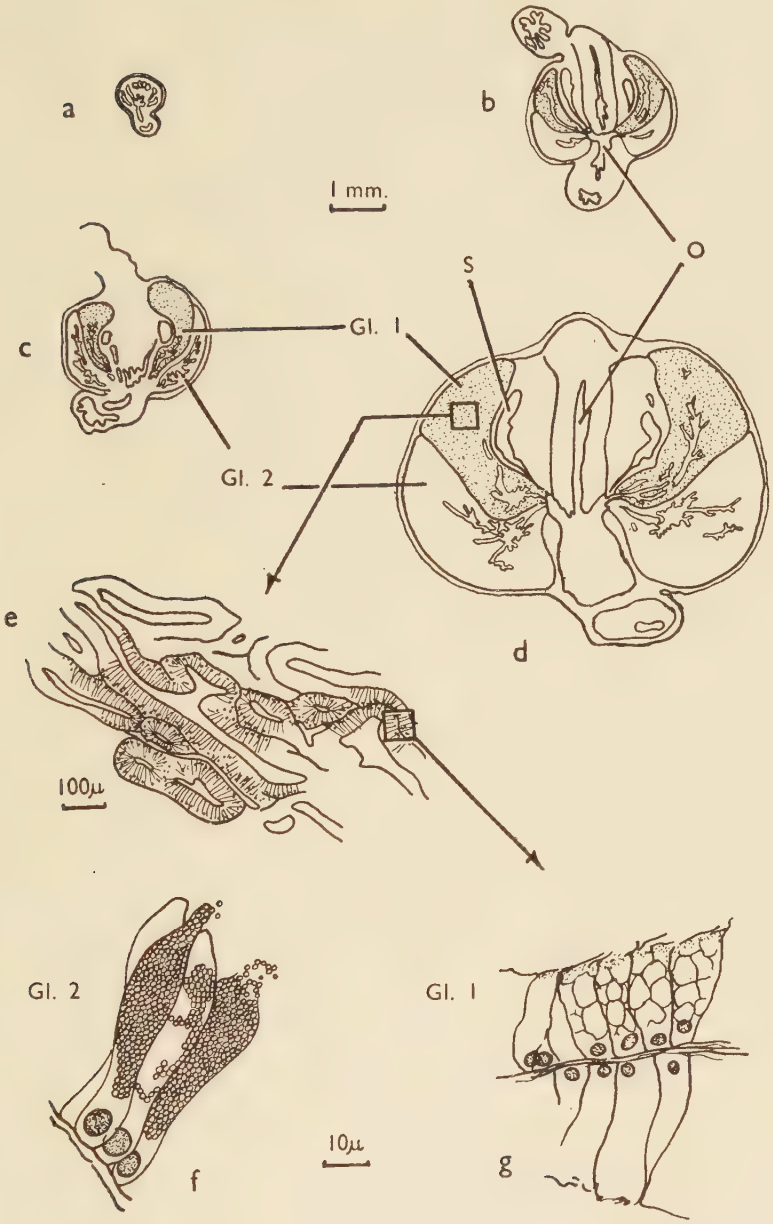

Fig. 8.—Structure of the oviducal glands and changes associated with enlargement of the optic glands and ripening of the ovary. (a) and (b), oviducal glands in unoperated immature controls of 37 gr. (EKL) and 610 gr. (C610). (c) and (d), precociously enlarged glands in animals with central brain lesions ; (c) from animal EK1C of 52 gr., forty-five days after operation and (d) from animal C27 of 890 gr. sixty-four days after operation. All these glands are cut in or near the plane of the oviduct and their radially segmented structure does not show ; they may be compared with fig. 6d drawn from an off-centre section in the same plane. (e), (f) and (g) show details of the structure of the enlarged gland of C27. o=oviduct, s=spermatheca, G1. 1 and G1. 2=secreting portions of the oviducal gland ; G1. 2 produces a strongly reducing substance in droplets, and has a gross structure similar to that of G1. 1.

before the glandular parts of the oviducal gland which presumably secrete the eggshell—and with the emptiness of the sacs in experimental animals kept isolated from other octopuses. When a female octopus that has just copulated is cut open, a spermatophore is found in the entrance to one or other of the oviducts (Racovitza 1894). It does not pass up the oviduct but is later discarded, the sperm presumably having in the meantime been attracted up the oviduct by some product of the walls of the spermathecae in the oviducal glands.

Pending a more detailed examination it seems likely therefore that the function of the oviducal glands is twofold. They store sperm, and they provide a hard shell to the egg ; it seems likely from their structure that eggs, in passing through them, are first fertilized and then coated with secretions that later harden to form the eggshell. Presumably the stalks of the eggs remain sticky for a while after passing through the glands and this permits their attachment to each other and to the surface from which they hang during incubation.

HORMONAL CONTROL OF SEXUAL MATURITY IN MALE OCTOPUSES

As already explained, the experiments upon which this account is based were made with female octopuses because enlargement of the ovary and its ducts following experimental treatments is so much more spectacular than the corresponding effects of induced optic gland secretion on the testis.

One reason for this is that the testis is never so great in proportion to the rest of the body as the ripe ovary ; another is the much earlier attainment of sexual maturity by males. Unoperated male octopuses weighing as little as 250 gr. frequently have spermatophores in their testis ducts, and all males of more than 400 gr. are, on this evidence, ripe. The optic glands of male animals of less than 1000 gr. are not, however, noticeably larger than those of females of similar size, and their histological appearance is identical. From this alone one might suppose that the condition of the testis was not dependent upon optic gland secretion. But experimental treatments causing enlargement of the optic glands also cause enlargement of the testis ; the mean testis weight both of animals with the subpedunculate lobe removed (data from fifty-four experiments) and of blind animals (forty-six experiments) was significantly greater than the mean testis weight of controls (eighty-four octopuses) throughout the 200-1000 gr. range (Wells & Wells 1959). Moreover a single experiment with a very small male octopus implies that, as in females, precocious maturation of the gonad can be brought about by treatments causing enlargement of the optic glands. This animal (EK1B) had a central lesion including the subpedunculate lobe. When killed forty-five days after operation the body weight was 79 gr. (the animal having nearly doubled in size since operation) and the optic glands large and orange ; the testis, with spermatophores in it, weighed 1·45 gr. This is a testis weight to be expected in control animals of from two to three times the size (Wells & Wells 1959) and is more than ten times the weight of the unripe testis from an octopus of 47 gr.—the next smallest animal to be measured.

It is possible that, unlike the ovary, the testis does itself produce a hormonal product since castration is followed by degeneration of the vas deferens

and spermatophore sac (Taki 1944); castration does not, however, affect regeneration of the hectocotylus (Callan 1939).

DISCUSSION

Evidence reviewed above shows that :

1. The onset of sexual maturity in *Octopus*, whether normal or precociously induced by the experimental treatments summarised in Figs. 9b and c, is preceded by enlargement of the optic glands.

2. If the optic glands are first removed, neither blinding nor removal of the subpedunculate lobe induce precocious ripening of the gonad as they would otherwise do.

3. Control of secretion by the optic gland is evidently entirely nervous, since unilateral lesions (including peripheral optic nerves section) induce enlargement of the optic gland on the ipsilateral side only.

4. Control of secretion is wholly inhibitory and there is no evidence for an excitatory nerve supply (See Fig. 9c).

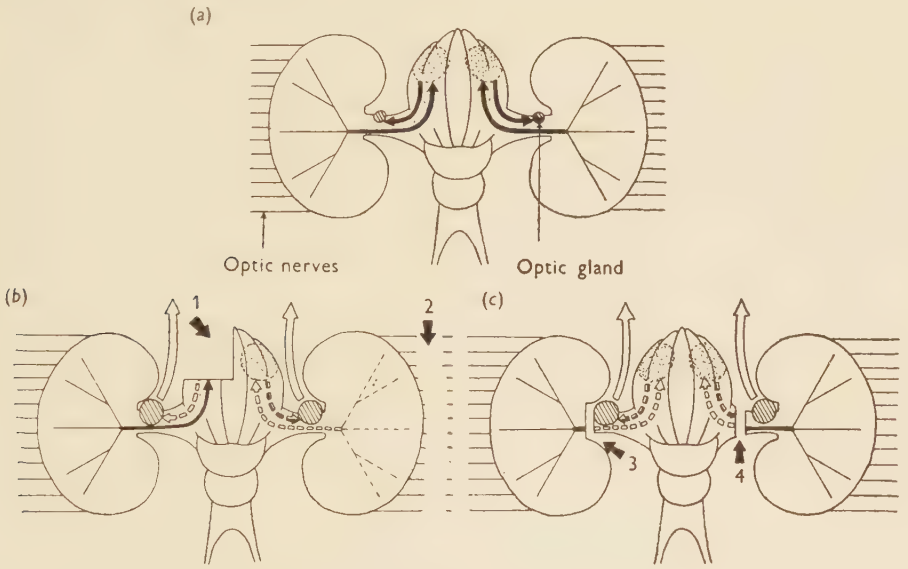

Fig. 9— A summary of the mechanism of hormonal control of sexual maturity in *Octopus*. (a) The situation in an immature, unoperated animal, where secretion by the optic glands is held in check by an inhibitory nerve supply, and ultimately by light. (b) shows two operations that allow the optic glands to secrete a product causing the gonad and its ducts to enlarge, being (1) removal of the source of the inhibitory nerve supply and (2) optic nerves section. (c) shows two further operations, (3) optic lobe removal and (4) optic tract section, that also lead to enlargement of the optic glands and gonads, thus eliminating the possibility that there is an excitatory innervation of the optic glands in addition to the inhibitory supply (from Wells & Wells 1959, *J. exp. Biol.* Vol. 36, p. 28).

5. Since the effects of peripheral blinding and subpedunculate lobe removal do not summate to produce a more rapid rate of enlargement of the gonad than the central lesion alone, the effect of blinding must be mediated through the subpedunculate lobe. The situation is summarized in Fig. 9a.

6. Experiments in which the subpedunculate lobe was removed from very young animals show that oviducts and oviducal glands become competent to react to optic gland secretion before the ovary. The histological changes taking place in these ducts closely resemble those occurring in the ducts of larger animals maturing normally or as a result of experimental procedures and it is concluded that optic gland secretion affects oviducts and oviducal glands directly, and not via an intermediate hormone produced by the ovary.

Clearly this control system has much in common both with the anterior pituitary-gonad control mechanism in vertebrates and with systems regulating the onset of sexual maturity in arthropods. The anterior pituitary, the optic glands, the X organ-sinus gland system of crustacea and the corpus allatum of insects are all regulated by efferent nerves arising in the highest centres of the central nervous system. All, moreover, serve in one way or another to delay the onset of sexual maturity until the animal is fully developed, though the details of the mechanism by which this delay is achieved vary considerably from one phylum to the next and within the Arthropoda. Thus cephalopods and vertebrates have in common that the gonad will not mature until the controlling gland secretes a necessary product, while the analogous glands of arthropods tend to prevent the too-early development of adult characteristics by producing an inhibitory hormone ; removal of the anterior pituitary or optic glands causes regression (or at least no further development) of the gonad while removal of the X organ-sinus gland or corpus allatum removes a brake on further development of adult characters. The four systems also differ in the way that secretion by the critical gland is controlled. In cephalopods control appears to be wholly inhibitory. In the higher vertebrates production of secretion by the anterior pituitary apparently depends upon a nice balance between an excitatory product carried in the portal blood system from the hypothalmus and an inhibitory factor, about which little is known beyond the fact that it is also hypothalamic in origin since hypothalamic lesions sometimes result in over-excitation of the gland and precocious sexual maturity (Harris 1955). In Crustacea the condition of the gonad is regulated by secretions from two different sources, an inhibitory secretion from the X organ-sinus gland system (Knowles & Carlisle 1956) and an excitatory secretion from the " Y organ " (developed from the excretory organ of the antennary or maxillary segment (Arvy, Echalier & Gabe 1956)) ; both of these glands appear to be regulated by excitatory nerve supplies. In insects secretion of the " juvenile hormone " from the corpus allatum depends upon the arrival of a neurosecretory product from the supraoesophageal ganglia (Wigglesworth 1940) ; after metamorphosis the same gland secretes a gonadotrophic hormone and release of this is governed by an excitatory centre in the suboesophageal ganglion as well as an inhibitory centre in the supraoesophageal brain mass (at least in some species—Englemann 1957).

In detail, then, there is little similarity in the modes of operation of the mechanisms controlling the state of the gonad in cephalopods, vertebrates and arthropods. Clearly these systems have been evolved quite independently in the three groups (and probably twice within the Arthropoda, insects and Crustacea

being in any event rather distantly related (Tiegs & Manton 1958)). In which case why the constant direct control by the highest centres of the central nervous system, parts that are otherwise concerned only with sensory integration and typically act only through intermediate centres?

A possible reason for this fundamental similarity of anatomical organisation can be discovered by considering the conditions under which selection might favour the evolution of mechanisms for delaying the onset of sexual maturity.

All other things being equal, selection will favour the animals that breed most rapidly, and act against the evolution of delay mechanisms. There are, however, clearly advantages in some sorts of elaboration that outweigh the disadvantages of a long development. Animals such as the higher vertebrates and the cephalopods have very well developed nervous systems that only become maximally efficient as a result of use, but the animals can profit by experience and adjust themselves to a wider variety of environmental disturbances than would be possible if they were wholly dependent upon genetically determined responses. Among arthropods the advantages compensating for development through a long series of larval instars are not to be found in additional flexibility of behaviour—intellect is not one of their outstanding features—but rather in the possibility of independent specialisation of larval and imaginal forms for distribution and food gathering. Here again there is a tendency for the stages finally produced to have relatively well developed nervous systems ; the final instars of arthropods are, like vertebrates and cephalopods, typically forms in which behaviour comes to depend upon distance receptors located on the head, a development that is necessarily linked with the evolution of higher integrative centres in the brain. Since selection must operate *before* the animals begin to breed, a direct association between the highest centres of the nervous system and mechanisms for delaying the onset of sexual maturity is therefore not altogether surprising, and since all nerve cells are potentially neurosecretory, control of the state of the gonad by secretions of glands arising out of the developing nervous system itself would appear to be inherently the most probable result of the interdependence of higher nervous development and delayed maturity.

In part, of course, the evolution of any such control mechanism must involve development of sensitivity by the target organ—either in the direction of inhibition by a product secreted or in the direction of increased dependence upon the product for development. As the situation in *Octopus* shows, the gonads in the two sexes may be differentially sensitive to the same secretion ; the testes of male octopuses slowly mature even when the optic glands are inactive, conditions under which the more secretion-dependent ovaries never ripen. Presumably this differential sensitivity is related to the much greater hazard presented by the onset of sexual maturity in female octopuses, and it would be interesting to consider differential sensitivity of target organs of other groups in this light. One would expect to find, for example, more rigid pituitary control of testis condition in male birds when bright plumage and elaborate sexual displays are associated with maturity—species in which the hazard of being conspicuous is selectively worth while only when the females are ripe too—than in cryptically coloured or relatively predator-free species.

SUMMARY

1. In cephalopods, the onset of sexual maturity is preceded by enlargement of the optic glands.

2. These are in turn controlled by an inhibitory nerve supply from the subpedunculate lobe in the supraoesophageal brain mass; cutting this nerve in immature animals initiates secretion by the optic glands and is followed by precocious maturation of the gonad.

3. Peripheral optic nerves section also initiates optic gland secretion and early maturation of the gonad, though at a slower rate than central lesions.

4. The effect of blinding and the effect of central lesions do not summate to produce an extra-rapid enlargement of optic glands and gonad, which suggests that the effect of blinding operates through the central control region.

5. Unilateral peripheral blinding or central damage affects only the optic gland on the side of the lesion, which implies that all stages in the control of optic gland secretion are nervous.

6. Enlargement of the ovary, whether normal or precociously induced, is accompanied by maturation of the oviducts and oviducal glands. The histological changes that occur appear to be identical throughout the size range (13-3320 gr.) of the octopuses studied, with the exception that in very small (<60 gr.) animals the ovary itself fails to respond to optic gland secretion. Since its ducts nevertheless develop normally it is concluded that these are directly dependent upon optic gland secretion rather than upon any product of the ripening ovary.

8. The glandular control system found in cephalopods is compared with analogous mechanisms regulating the onset of sexual maturity in vertebrates and in arthropods, and factors tending to produce a convergent evolution of mechanisms for delaying the onset of sexual maturity in the three groups are discussed. The differential sensitivity of the target areas of the two sexes of *Octopus* is cited as an instance where selective pressure has lead to a more rigid dependence upon the controlling secretion in the female, where the hazards of sexual maturity are much greater than in the male.

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MOULTING CYCLES IN CRUSTACEA

BY

D. B. CARLISLE

Marine Biological Association of the United Kingdom, Plymouth

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CLASSIFICATION OF THE STAGES OF THE MOULT CYCLE

The abbreviated life history of insects has led insect physiologists to pay little regard to the cyclical nature of the moult in arthropods. In Crustacea, on the contrary, many of the species continue moulting long after sexual maturity is reached and the cyclical nature of moulting is most apparent. The differences in approach of the two groups of workers may be seen in the emphasis that has been placed upon the stages of the moult cycle in Crustacea, while little or no attention has been paid to the various stages through which an insect passes as it moults. In Crustacea two schemes of classification of the moult cycle have proved acceptable from different points of view. For practical purposes that proposed by Drach (1939) is the most convenient, but certain theoretical considerations not apparent in his scheme make that of Carlisle & Dohrn (1953) more suitable for the purposes of discussion. In this scheme the moult cycle is divided into the following stages :

Stage 1 : PROECDYSIS or premoult. During this stage the animal is preparing to moult, most noticeably by the removal of calcium from the exoskeleton (in those forms which have a calcified shell) ; in the more highly calcified forms parts of the old shell become brittle and may flake off. With this is correlated a rise in the blood-calcium level. In all forms, whether calcified or not, the epidermis becomes heightened and a new exocuticle is laid down between the old shell and the epidermis. Just before the moult the two cuticles, old and new, are completely separate.

Stage 2 : ECDYSIS, moult or exuviation. This is the short period of the cycle while the animal is actually shedding its exoskeleton.

Stage 3 : METECDYSIS or postmoult. This is the period when the new exoskeleton is hardening and the animal is returning to normal in its physiological condition. In the earlier stages of metecdysis the animal is not yet feeding and, indeed, cannot support its weight on its legs. As the shell hardens it begins to feed. During metecdysis the larger Crustacea tend to shun the more exposed positions, and to skulk in holes and crannies.

Stage 4 : intermoult. This is the stage of normality to which the altered conditions of the other stages may be referred. The shell is hard, the animal is feeding, blood-calcium is low and growth is at its height. The intermoult may be of two types :

(a) ANECDYSIS is a long period of " rest " (in the sense that we talk of a " resting " nucleus) between the conclusion of one metecdysis and the beginning of the next proecdysis. This is found in animals which moult seasonally, e.g. *Cambarus*, *Uca*, *Maia*.

(b) DIECDYSIS is a short period during which a metecdysis passes imperceptibly into the succeeding proecdysis. This is found in animals which moult all the year round, e.g. *Lysmata*, *Ligia*, *Leander*.

Some crabs, e.g. *Pachygrapsus*, *Carcinus* (at least the Plymouth population of *C. maenas*), show a series of moults during the summer months, separated by diecdyses, the yearly set of moults being divided by anecdyses during the winter (see Hiatt, 1948). We may suspect *a priori* that the mechanism governing anecdysis may be different from that for diecdysis.

Some crustaceans, e.g. *Cancer*, *Homarus*, can apparently go on growing and moulting indefinitely. Specimens of both species weighing 20 kg have been taken in British waters in the last ten years. One lobster of this weight measured 120 cm from the tip of the rostrum to the tip of the telson, while a crab landed at Plymouth was 91 cm in carapace breadth. On dissection it was found to be in proecdysis, so that if it had lived to moult it would presumably have increased in weight by about 10 kg and in carapace breadth by some 15 cm. By contrast, other species cease to moult and to grow at some more or less definite size or number of moults. They then enter upon a terminal anecdysis (Carlisle, 1957) and never, except after experimental interference, enter upon another proecdysis as long as they may live. In some of them the last moult is also, as in the insects, the moult of sexual maturity ; such include the spider crab, *Maia* and the copepod *Calanus*—perhaps the most abundant animal in the world. *Calanus finmarchicus* has six instars as a nauplius larva before it metamorphoses to a copepodid, then five instars as a juvenile copepodid ; the last moult is the moult of puberty, followed by sexual maturity, and corresponds to the last moult of the insects. A second class of Crustacea possessing a terminal anecdysis may have several moults after sexual maturity. *Pachygrapus*, *Carcinus*, *Leander* and *Portunus* fall into this class. Male *Carcinus* appear to undergo ten or eleven moults after the moult of puberty before entering into terminal anecdysis (Carlisle, 1957). In *Callinectes* the last moult in the female is the moult of puberty, so that the female falls into the first class, while the male may undergo more moults after sexual maturity, falling into the second class.

In the scheme of classification of the moult cycle adopted by Drach (1936, 1939) emphasis is laid upon the various points whereby the stage of the moult cycle may be determined in the living animal. For practical purposes this is of course of first-rate importance. He divided the moult cycle of *Cancer* into five primary stages which he denoted by letters, and into twelve distinguishable substages, denoted by subscript numerals :

Stage A : immediately after moulting ; equivalent to the first part of proecdysis.

A_1—during which the exoskeleton is a soft membrane, the animal cannot stand on its own feet or support its weight, water is still being absorbed and the animal has no stable weight.

A_2—the animal can now stand, but is still soft. The water content (in *Cancer*) is about 86 per cent.

Stage B : the main period of hardening of the shell and of calcification in those species which have a calcified shell ; equivalent to a middle period of proecdysis ; feeding has not yet begun ; the water content drops as calcium is absorbed and deposited.

B_1—the meropodite and propodite can be bent without cracking ; water content 85 per cent.

B_2—the meropodite and propodite crack if bent ; water content 83 per cent.

Stage C : the longest part of the cycle, when the animal leads a normal life ; the carapace is hard and the animal feeds. Water is gradually replaced by tissue.

Stages C_{1-3} are equivalent to the last part of metecdysis, C_4 to anecdysis in the scheme of Carlisle & Dohrn.

C_1—the opposite broad faces of the legs will give without breaking under finger pressure ; water content 80 per cent.

C_2—the opposing faces of the walking legs will still give slightly under finger pressure, but will crack if greater pressure is applied ; water content 76 per cent.

C_3—the integument of the legs is completely rigid, but a slight translucency of the lateral and frontal portions of the carapace indicates that calcification is still not complete in these regions ; water content 68 per cent.

C_4—this is by far the longest stage in *Cancer* and is commonly referred to as the intermoult period. The shell is completely calcified and is now lined by an inner membranous layer which is adherent to it ; water content 61 per cent.

Stage D : the stage of preparation for the following moult, during which calcium is resorbed from the old shell, new cuticle is secreted and other changes take place. The animal ceases feeding at some time during this stage. The water content is about 60 per cent. This is equivalent to proecdysis.

D_1—the first signs of the coming moult are seen in the precocious formation of new setae in the cores of the old ones. This phenomenon, first observed by Braun (1875), is readily observed under the binocular microscope or with a hand lens and is of great importance in determining the incipience of moulting.

D_2—the stage of secretion of the new pigmented layer of the integument. This is difficult to observe in the intact animal, but quite obvious upon dissection.

D_3—very evident resorption of calcium and other substances from the shell, often accompanied by cracking of parts of the shell, which may flake away. The new integument becomes completely detached from the overlying old shell.

D_4—complete resorption of the shell along the line of dehiscence until the carapace may gape along this line.

Stage E : ecdysis.

One disadvantage of this scheme is immediately apparent—it is necessary to modify one's criteria for each new species studied. The system can, then, have no general validity. But so long as it is recognized that the stages are arbitrary this scheme, variously modified, can be of great value in practical studies of the moult cycle, especially if the broad classifications are then related to the stages of the scheme of Carlisle & Dohrn. One or two of the obvious modifications which may be incorporated into classifications of the moult cycle of other species may be mentioned here : in non-calcified forms stage B is characterized by a further slight increase in water content rather than a drop since no calcium is absorbed and the animal is still not forming new tissue ; many species begin feeding during stage B ; non-calcified forms show no flaking of the carapace during stage D_3 ; the water contents will be very variable from species to species. Nevertheless, the practical value of this division of the moult cycle is evident, and it has been used in many studies of moulting in various crustaceans. Hiatt (1948) added to its usefulness by defining stage C_4T, by which he denotes the stage here called terminal anecdysis.

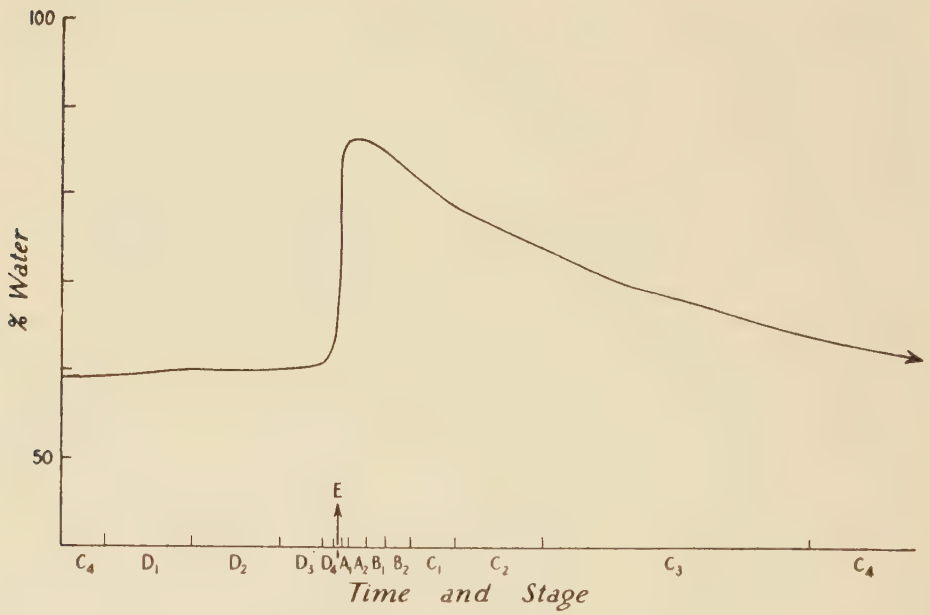

Fig. 1.—Smoothed curve representing the percentage water content of *Carcinus maenas* at various stages in the moult cycle, and relative lengths of the stages in this species, reduced to a standard temperature. The results summarize the data from about 400 animals.

Either or both of these schemes may readily be extended to insects, and, indeed, in a current study of the comparative endocrinology of moulting in Crustacea and insects, Dr Peggy E. Ellis and I have found it useful so to classify the moulting cycle of locusts. Briefly, the beginning of proecdysis may be distinguished by the appearance of a new pigmented layer under the integument (D_1). In stage D_2 the new layer begins to separate from the old cuticle, but

the muscles still remain attached ; the animal is still feeding. Feeding ceases during D_3, when the muscles lose their attachments to the old shell ; in the larger hoppers pressure of the finger nail on the pronotum will cause the pattern to shift as the underlying pigmented integument moves relative to various unpigmented spots in the outer shell. The line of dehiscence gapes during D_4, and on dissection the lining of the trachea can be pulled out with ease. The first stage of metecdysis (A_1) is represented by the interval when the animal cannot stand on its feet without causing permanent deformation of the legs as they bend under the weight ; in the fledgling it is also the time during which the wings are inflated. During A_2 the carapace is hardening rapidly and melanization of the darkly pigmented areas is proceeding but not yet completed. Feeding begins during stage B_1 and melanization is complete at its commencement ; the third femur can be bent without permanent kinking. During B_2 a permanent kink results if the third femur is bent. Stages C_{1-4} are telescoped into a brief diecdysis in the hoppers, but in the adult, which is, of course, characterized by a terminal anecdysis, C_4T is distinct from the other substages of stage C which merely represent the final stages of the hardening of the cuticle and the end of metecdysis. It is interesting to note that in the locust *Schistocerca*, individuals which are in adult diapause proceed no further with the hardening of the shell than C_3, until the diapause is broken (Ellis & Carlisle, unpublished). Stage C_4T is characterized by the hardness of the pronotum which is such that a sagittal cut with a sharp scalpel results in fragmentation so that a clean cut is impossible : this is the fully hardened condition. During stage C_3 the pronotum and head may be bisected cleanly. This classification of the moult cycle has made it possible to correlate changes in the histology of endocrine organs with the moult cycle and has led to greatly simplified experimental techniques.

ENDOCRINE CONTROL OF THE MOULTING CYCLE

The greater part of the experimental work on the endocrine control of the moulting cycle has been devoted to proecdysis and the preparation for the moult; little or nothing has been done to investigate metecdysis, and there has been a tendency to regard anecdysis as a " resting " period. In fact anecdysis represents, in the larger species at least, the major part of the life and the period of maximal growth. But before we turn to a consideration of the endocrine control of moulting cycles it would be useful to enlarge upon the question of growth. It is important in arthropods to distinguish growth from mere increase in size. A crustacean increases in size by a succession of moults, but it is wrong to regard the process of ecdysis as the stage during which growth takes place, as has been done so frequently. At each normal moult the shell is cast and the animal swells by absorption of water (or of air in insects and terrestrial arthropods generally). The outer layers of skin are then hardened. The increase in size at moulting is, then, merely an increase in the wet weight of the body ; in insects it is not even that for an insect loses weight on moulting, despite the increase in body volume. Throughout the rest of the period between successive moults, excluding the periods at each end when the animal is not feeding, the water (or air) is replaced gradually by new tissues, either by

the swelling of old cells or by the formation of new ones. In my view it is more convenient to regard this process, involving protein synthesis and mitoses, as a better index of growth than increase in size. An arthropod, then, may grow without increasing in size ; and we must concede that in most insects growth continues long after the last moult.

It has been known for over fifty years that the eyestalks influence moulting and it is now twenty years since it was first suggested that this influence was hormonal. The greater part of our knowledge of the endocrine influences on the moulting cycle derives from experiments upon the eyestalk. The structure of the endocrine systems of the eyestalks has been elucidated in a number of species, most recently in *Pandalus borealis* (Carlisle, 1959*a*). A general account of a number of species is to be found in Carlisle & Knowles (1959). In 1939 Brown & Cunningham, Abramowitz and Hanström all found that removal of the eyestalks in various species of Reptantia led to precocious moulting. This has been confirmed repeatedly in the succeeding twenty years in numerous species, but it cannot, nevertheless, be taken as a general rule. It is only under certain special conditions that these two events are causally related. As with so much biological research it has been a very happy accident that these special conditions were realized in these early experiments. It if had not been so, the study of the hormonal control of crustacean moulting cycles and metabolism generally would not have received the impetus which it has, and our knowledge of invertebrate endocrine systems would have been greatly retarded.

It is now generally held that during anecdysis the X organ-sinus gland complex of the eyestalk is secreting a so-called moult-inhibiting hormone which prevents the initiation of proecdysis. The most complete study of this problem is that by Passano (1953) and his results have since been substantiated in many species. So long as the animal is receiving an adquate supply of this hormone proecdysis is inhibited and the animal remains in anecdysis. There is some evidence that the same hormone, or one which is so far biologically indistinguishable from it, is produced within the cerebral ganglion, presumably in the neurosecretory cells there. The hormone is also to be found in the eyestalks of crabs and prawns during diecdysis (Carlisle, 1957, 1959*b*), but, at least in *Carcinus*, there is a very much lower titre during diecdysis than during anecdysis. I have found (unpublished results) that the termination of anecdysis in *Carcinus* is associated with a rapid drop in the titre of the moult inhibiting hormone in the eyestalks, this titre dropping simultaneously with the rise in blood-calcium level which is one of the first signs of proecdysis. On the experimental level it is one of the oldest established phenomena in crustacean endocrinology that removal of the eyestalks from well-fed reptantians in anecdysis leads to the initiation of proecdysis and to subsequent precocious moulting. In the more sophisticated experiments of Passano (1953) and others this result has followed removal of the X organ-sinus gland complex. Indeed, in *Carcinus*, eyestalk removal or removal of the X organ-sinus gland complex from males in terminal anecdysis is followed by proecdysis and up to four moults in rapid succession, with the production of giant crabs. It is noteworthy that in this species the titre of moult-inhibiting hormone in the eyestalk

is much higher during terminal anecdysis than during an anecdysis earlier in the life history (Carlisle, 1957).

It is sufficiently established that during anecdysis the moult-inhibiting hormone of the eyestalk prevents the initiation of proecdysis until the due moment arrives. Nothing is known of influences which lead to the cessation of production of this hormone at the appropriate moment in the life history. We may, however, suppose that this may result from different stimuli in different species, in the way that the initiation of the breeding season in mammals, or the termination of the diapause in insects, may be induced by different environmental stimuli in different species. Altering daylength, rising or dropping temperatures and seasonal abundance of food may all be suspected as possible stimuli in species which have a seasonal moult, while internal metabolic mechanisms, such as low water content, high fat or protein reserves or other more subtle mechanisms may play their part in species which may moult at varying times throughout the year.

Nor do we know much about the mechanism of action of the moult-inhibiting hormone. Various pieces of information lead us to believe that the hormone is not acting directly upon the tissues which are immediately concerned in moulting. It would seem more probable, in the light of such knowledge as we have on this point, that the hormone exerts its influence upon the Y organ described by Gabe (1953), while it is certain that in the absence of the secretion of the Y organ proecdysis cannot begin. Echalier (1953, 1954) found that bilateral ablation of this organ from crabs in any stage of the moult cycle up to the beginning of proecdysis caused a halt in anecdysis. He kept crabs for more than a year in permanent anecdysis. If the operation was performed after the beginning of proecdysis, this stage, ecdysis and metecdysis proceeded normally but the animal then halted in anecdysis, never continuing to another proecdysis. It is apparent that the secretion of this gland is needed for the initiation of proecdysis, but not seemingly for any other stage in the moult cycle.

It is possible, or indeed, I would say it is probable that the Y organ is homologous with the prothoracic gland of insects. Functionally there is a very close similarity (though it is not on this that the possible homology depends). Both glands appear to be stimulated to secrete by hormonal control emanating from the protocerebrum : the secretion of both is needed for the initiation but not for the continuation of proecdysis ; in most pterygote insects the prothoracic gland disappears after the last moult : in the spider crab *Maia* the Y organ degenerates after the finale moult (Carlisle, 1957). Skinner & Karlson (1959, and personal communication) have shown that the secretion of the two glands is chemically very similar and that the insect secretion is active upon shrimps and *vice versa*. I have found that while the crustacean extract is about 1000 times less active than the hormone prepared from silkworms, when assayed upon blowfly larvae, the ratio of activities is reversed when the assay is performed upon prawns (unpublished work, partly in collaboration with Karlson). In other words, although the secretions are very similar each is far more active upon its own class of animals.

The end of anecdysis, then, is brought about, on the hypothesis we are adopting in this paper, by the cessation of the secretion of the moult-inhibiting

hormone by the X organ-sinus gland complex of the eyestalk ; this stimulates the Y organ to discharge its secretion and provoke the initiation of proecdysis. What hormonal influences can we find bearing upon the progress of proecdysis once it has begun? Carlisle & Dohrn (1952, 1953) found that eyestalk removal in the prawn *Lysmata* (which has no anecdysis) was followed by a decreased proportion of animals moulting, while injection of eyestalk extracts was followed by the reverse effect. They termed the responsible principle, which they believed to be hormonal, a moult-accelerating hormone. Subsequent analysis has shown that this substance is produced in the X organ-sinus gland complex, but is stored in the sensory pore X organ, not in the sinus gland, where most hormones of this complex are stored ; and that it exerts its effects solely upon proecdysis, being without effect at other stages in the moult cycle. Its general action seems to be to regulate the coordination of the various processes which together constitute the preparations for the moult. It can also be found in extracts of the brain. In crabs, indeed, it can only be found there, for the X organ-sinus gland complex of crabs has a very reduced sensory pore X organ component, and the eyestalks are without a detectable titre of the moult-accelerating hormone. In crabs, therefore, removal of the eyestalks can only result in the removal of the moult-inhibiting hormone, and not of any other hormone effecting the moult rate. This operation in crabs (and in crayfish) can thus only result in an accelerated moult rate in the population at large, if it has any effect ; it cannot under any circumstances lead to a fall in the moult rate. In prawns, however, where the eyestalks contain both a hormone which inhibits the initiation of proecdysis and one which speeds proecdysis once this has started, eyestalk removal may lead to an overall increase in moult rate in the population, or to an overall decrease, depending upon the balance of individuals in different stages of the cycle and upon the balance of the production of these two hormones in the eyestalks and in the brain. Different populations of the same species even may show quite opposite effects under identical circumstances (Carlisle, 1959*b*).

Despite this divergence between different groups of decapods, the principle remains the same—neurosecretory cells of the protocerebrum (either in the eyestalk or in the central portion of the brain) during proecdysis secrete a hormone which coordinates the processes of proecdysis, and if the amount available to the animal is reduced, then proecdysis takes longer and there is a greater proportion of failures of the moulting process itself.

There remains one more hormonal influence bearing upon the moult cycle—that which regulates the uptake of water at the moult itself. This is a product of the eyestalks, found, like the moult-inhibiting hormone, in the sinus gland, but apparently not identical with it. I have shown (Carlisle, 1956) that in the absence of this hormone too much water is taken up at the moult—sometimes as much as three times the volume of the pre-moult animal. It is possible also that it is concerned in the water and salt balance of the animal between moults, besides regulating the uptake of water during ecdysis.

Little or nothing is known about any hormonal regulation of metecdysis, though there has been some suggestion that the hardening of the shell is controlled to some extent by the eyestalks. Nor is much known about the

control of the differences between anecdysis and diecdysis in those species in which both stages occur. It is certain that anecdysis is not possible in an animal which lacks eyestalks, and the endocrine status of the eyestalk is different in the two stages (see Carlisle, 1957), but no picture has emerged of their determination. Nevertheless they are as dissimilar as anoestrus from dioestrus.

That growth and metabolism in Crustacea are constrained by the necessity for a series of moults has led to a cyclical nature being imposed upon many other activities of the body which in other animals are not bound up with growth. Oxygen consumption, respiratory quotient, calcium metabolism, metabolism of sugar and glycogen (needed as the precursors of chitin), proteins and nitrogen, lipoids and carotenoids, all show cyclical variation with the moult cycle (see Carlisle & Knowles, 1959). But it is not with these that I wish to concern myself in this discussion. It requires but little thought to see that these processes bear more or less directly upon the moult process itself and hence must to some extent be governed by the moult cycle. No experimental investigation, moreover, devoted to the elucidation of the hormonal control of any of these processes has distinguished between such control and the basic control of the moult cycle. It is impossible, for instance, to say at the present whether the effect of removal of the X organ-sinus gland complex upon respiratory metabolism is a result of interference with the moulting hormones of this complex, or whether a different hormonal system is involved. But other processes of the body have been investigated, which do not of necessity bear directly upon the moult cycle, and it is the interplay of such processes with moulting that I wish to explore now.

MODIFICATIONS OF THE MOULTING CYCLE

It is not at first necessarily obvious, for example, that the maturation of the gonads and reproductive system will involve interference with the moult cycle. Yet the organs of copulation, the special structures required for brooding the eggs and the pigmentation which characterizes an egg-bearing prawn all involve the integument and shell, and hence can only appear or be modified at a moult. And it is known that these characters are under hormonal control by the gonads, the vas deferens gland or the endocrine complex of the eyestalk (see discussion in Carlisle & Knowles, 1959). It is evident, therefore, that those aspects of the effects of these hormones which result in some altered morphology of the animal after a moult, can, from one point of view, be regarded as modifications of the normal moult cycle. The influence of the hormones must be exerted upon the tissues which are preparing for the forthcoming moult during proecdysis, with perhaps a brief extension of action during the moult itself and the first part of metecdysis. A gross increase in the size of the appendix masculina, or of the oviferous hairs, must be prepared during proecdysis, to find expression at the succeeding moult.

So far, then, as their influence upon the integumental characters is concerned such hormones may conveniently be regarded as modifiers of the normal moult cycle, and the special moults which result as modifications of "normal" moults. Such special moults as the prepuberal moult (when the sexes first be-

come distinguishable externally), the moult of puberty, the moult of copulation of the female crab (when she exudes an attractive pherormone and produces the brooding characters) and the critical moults of sex-reversal in the protandric hermaphrodites may be regarded in this light, as modifications of the more normal moult. And it is, of course, then obvious that the moult may be modified in more than one way at once. Thus the moult of puberty in a female crab may or may not be also a moult of copulation, and moults of copulation may occur long after puberty.

There is some evidence that in the absence of modifying influences a fresh moult will restore the simpler condition found before the onset of one of these special moults. Thus the hormonal influences from the ripening ovary are needed at each moult which is to be followed by the appearance of the brooding characters and ovigerous hairs. Two or more successive moults may be accompanied by the continuation of these brooding characters, but only so long as the necessary hormone from the ripening ovary is being supplied (either naturally or by injection). Once this is withdrawn the next moult will see a return to the condition nearer the non-sexual with loss of the pre-existing brooding characters. Many of the anomalies which have muddled our thinking on parasitic castration may be resolved if we think instead in these terms. The morphometrics of a crab which has been parasitized are those of the pre-puberal animal—male and female have both the proportions of the non-sexual individual. That the female proportions diverge much less than those of the male from this condition has led to the belief that the parasitized male approximates to the female condition. It does no such thing—it approximates to the non-sexual condition. Preliminary assays of hormonal content of parasitized crabs have failed to reveal any traces of any of the sexual hormones which serve in the normal animal to modify the moults towards sexual development. I incline to the opinion, that so far as the integument is concerned, the major effect of the parasite (it has of course profound effects on the metabolism, but it is not with these that we are concerned now) lies in suppressing the production of those hormones which modify the moult so that any succeeding moults will be unmodified and show a return to the ontogenetically earlier condition of the integument—the pre-puberal non-sexual condition.

If we regard each moult as basically similar, with special modifying influences intervening in some of them, then we may regard the increasing complexity of the life history of the higher arthropods as analagous to the increasing complexity of the body structure of metamerically segmented animals. The primitive metameric animal possesses a segmental body in which each segment is the same as each other segment. With increasing complexity of structure these similar segments take on special characters of their own, not shared by other segments : each segment of the vertebrate head, for example, is different from each other segment. The specialization and differentiation of the various segments reaches its height in the birds, mammals, crabs and insects. Similarly, each moult seems homologous with each other moult and primitively like it. The increasing complexity of the life history has led to each moult taking on a special character and becoming different from the preceding and succeeding moults in the higher arthropods, thus masking

the basic cyclic nature of moulting and leading students to regard each moult as an event instead of part of a repetitive cycle.

A COMPARISON WITH VERTEBRATES

In conclusion I should like to mention two ways of some significance in which the endocrine system of Crustacea differs from that of vertebrates, one structural and one functional. The functional difference lies in the preponderance of inhibitory hormones in crustaceans, hormones whose most obvious effect is to inhibit the start of some activity. The moult-inhibiting hormone, the water balance hormone and the hormone which inhibits vitellogenesis are examples. Future experimental analysis may show this inhibition in a different light, but so far as our knowledge goes at the present I should incline to believe that it is a natural corollary to the markedly cyclical nature of crustacean metabolism. In the realm of nervous control of cyclical activity, e.g. heart-beat, we are used to thinking in terms of complementary inhibitory and stimulatory nerves, and in this allied field of hormonal coordination of function it seems not unreasonable to find the same antithesis. The second difference from vertebrate endocrine systems that I should like to mention lies in the development of the majority of the crustacean endocrine glands from nervous tissue, nervous tissue, moreover, which still retains the structure of nerve cells and axons and probably also the functional activity of neurones. Indeed, of all the organs in the crustacean body which are known to secrete hormones only two are not nervous in origin. In the remainder the hormone appears to be produced in the nerve cell body, transported down inside the axon to be stored in swollen nerve endings which terminate against the blood spaces, and there released into the blood. The organs consisting of swollen nerve endings in contact with the blood have been named neurohaemal organs (Carlisle & Knowles, 1953) and the total endocrine system of this nature represents a group of motor neurones whose effector is this neurohaemal organ, on the near view, or the target organ of the hormone on the long view.

It may be this close association between the nervous and hormonal systems of the body in Crustacea which has led to the close parallel of development between the two, with inhibitory as well as stimulatory hormones and nerves. And the liaison between the two systems is perhaps closer than in vertebrates, where the pituitary seemingly acts as the sole link.

SUMMARY

The act of moulting in arthropods must not be considered as an isolated event but as the most spectacular moment in a continuous cycle of metabolic activity. Each moulting cycle is basically like the one before it and many of the stages have now been shown to be under hormonal control. Secretion of the ' moult-inhibiting hormone ' by the endocrine complex of the eyestalk promotes the formation of new tissues and prevents the Y organ from secreting more than marginal amounts of its hormone. Once this eyestalk hormone ceases to be secreted into the blood stream the Y organ releases a single ' dose ' of a moult-promoting hormone which initiates the preparations for the moult, which, once under way, are coordinated by a moult-accelerating hormone from

the X organ. The amount of water taken in at the moult itself is regulated by a further eyestalk hormone. Many other hormones act upon particular moults and modify them in some way, acting mainly upon the stage of proecdysis and making the succeeding moult in some way unique : the moult of puberty and the moult at which a female becomes furnished with the brooding characters are examples. In the same way that the originally similar segments of the vertebrate body have been progressively and uniquely modified, in for example the head, so the originally similar moults of arthropods have been modified, till we have the startling difference between the larval and imaginal moults of Lepidoptera.

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